NATIONAL

E74-21268

BOSTON UNIVERSITY

GRADUATE SCHOOL

Dissertation

THE ACTION OF CYANAMID AND ITS RELATION TO CREATIN AND CREATININ

by

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(S.B., Yale University, 1932)

submitted in partial fulfilment of the

requirements for the degree of

Doctor of Philosophy

1938

CONTENTS

Introduction

Chemistry of cyanamid and related compounds

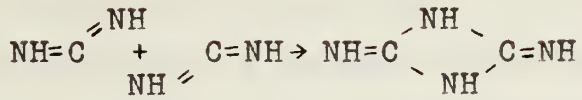
The first appearance in the periodical literature of an extensive study of the chemistry of cyanamid and its derivatives as well as their toxic effects is the work of Stritt (1909). Kalkstickstoff and Stickstoffkalk are the two commercial preparations of calcium cyanamid made in Germany. The essential difference between these two compounds is the presence of 6.5 per cent chlorine as impurity in the latter, with none in the former. They are grey-black substances, granular or powdered and only moderately soluble in water. $N{\equiv}C{-}N{=}Ca$ represents the structural formula of both of these.

There are several other compounds; namely, a monobasic calcium salt of cyanamid, with the formula, $(CNNH)_2Ca$: a dibasic calcium salt, $CNN(CaOH)_2$ plus $5H_2O$; a calcium carbonate compound of cyanamid, $(C_2N_2)_2Ca$; dicyandiamid a polymer of cyanamid, $C_2N_4H_4$; and finally dicyandiamidin, $C_2N_4H_6O$.

The monobasic calcium salts are unstable and will not be considered in this discussion. The calcium carbonate compound of cyanamid will not be described, inasmuch as it is of infrequent occurrence.

Cyanamid may be considered as a nitrile of carbamic acid, with the formula, $N{\equiv}C{-}N{<}^{H}_{H}$, or as a carbodiimid, $NH{=}C{=}NH$. From its relation to urea and its ready polymerization, which will be discussed later, one might be

tempted to favor the second of these formulae.



cyanamid dicyandiamid

This equation works satisfactorily with cyanamid expressed as the carbodiimid. However the evidence is insufficient to establish definitely the correct formula.

Preparation of calcium cyanamid and cyanamid

Calcium cyanamid is prepared by heating calcium carbide and nitrogen in the electric furnace.

Cyanamid can be synthesized either from calcium chloride and potassium cyanate, (Stritt 1909) or from cyanogen chloride and ammonia. It can also be prepared by the reaction between mercuric or lead oxide and thio-urea, or from technical calcium cyanamid after removal of the calcium as an insoluble salt,(Beilstein 1921).

The preparation from calcium cyanamid involves the precipitation of the calcium as calcium oxalate by the addition of oxalic acid, as calcium sulphate by the addition of aluminum sulphate, or sulphuric acid (Baum 1910), or by its reaction with carbon dioxide to form calcium carbonate (Osterberg and Kendall 1917). After filtration the procedure in all methods is to concentrate the solution of cyanamid to dryness. It is necessary to keep the temperature below 44°C in order to prevent the polymerization of

cyanamid to dicyandiamid. For that reason it is best concentrated in vacuo. The concentrate is then dissolved in ether in which cyanamid is soluble and dicyandiamid is insoluble. The cyanamid is finally recrystallized from ether.

Properties of cyanamid and related substances

Pure cyanamid is a white, hygroscopic, crystalline material easily soluble in water, alcohol, and ether. It is volatile in steam, and slightly soluble in carbon disulphide, chloroform, and benzol. It melts at 44°C according to the International Critical Tables (1930), or at 40°C according to Stritt and other workers, who probably did not have pure cyanamid.

Aqueous solutions of cyanamid are neutral to litmus (Hesse 1921). One gram is neutralized by 0.6 cc. of 1/10 N NaOH, with phenolphthalein as indicator (Stritt 1909).*

Acid hydrolysis with sulphuric, phosphoric, hydrochloric or nitric acids converts cyanamid mainly into urea. Excess dilute sulphuric acid transforms cyanamid into urea, dicyandiamid, and dicyandiamidin. Acetic acid changes it into ammonium acetate and urea. Thio-acetic acid converts it into thio-urea. Treatment with hydrogen sulphide results in the formation of thio-urea. Potassium hydroxide converts it into urea, while ammonia, on the other hand, transforms it into guanidin.

Stable metallic cyanamids or salts of cyanamid are

*In the writer's experience, one gm. of cyanamid (99 per cent pure) was neutralized by 0.12 cc. of 1/10 N NaOH.

easily formed by interaction of cyanamid with salts of sodium, potassium, calcium, barium, magnesium, silver, mercury, copper and lead (Heilbron 1934).

The silver salt of cyanamid is bright yellow, soluble in dilute nitric acid, but insoluble in ammonia. The copper salt is brown-black, soluble in acids and ammonia (Stritt 1909).

The polymerization of cyanamid to dicyandiamid, and tricyantriamid (melamine) occurs readily when aqueous solutions of cyanamid are allowed to evaporate or are heated above room temperatures. At 150°C it forms mostly dicyandiamid and partly melamine and ammonia. Cyanamid also reacts with guanidin or its salts forming biguanidin. Reduction with hydrogen results in the formation of methylamine and ammonia.

Dicyandiamid forms a white mass of rhombic crystals, melting at 207°C, moderately soluble in water and alcohol, soluble in hot water,and practically insoluble in ether (Stritt 1909). Its formation from cyanamid has already been described. On heating it forms melamine and ammonia. Hydrogen reduces it to guanidin and methylamine. It forms salts with many metals (Heilbron 1934). Silver nitrate precipitates the white silver salt of dicyandiamid from aqueous solution. This silver salt is difficultly soluble in cold water, slightly soluble in nitric acid, and easily soluble in hot water. The formation of the silver salt of

dicyandiamid has been made the basis of a quantitative determination of dicyandiamid in calcium cyanamid (Beilstein 1921).* Dicyandiamid can be detected also by boiling a solution for several hours with dilute acetic acid and then adding sodium hydroxide and copper sulphate. This treatment results in the formation of rosy-red copper dicyandiamidin (Beilstein 1921).

Dicyandiamidin is easily formed by the treatment of cyanamid or calcium cyanamid with an excess of dilute sulphuric acid. It forms stable salts with mineral acids and acetic acid. Its salts can be demonstrated by treatment with copper sulphate and sodium hydroxide to form the typical copper dicyandiamidin (Beilstein 1921).

The quantitative determination of cyanamid

The quantitative determination of cyanamid is based on its property of forming a yellow precipitate with silver nitrate. This salt is insoluble in ammonium hydroxide but soluble in nitric acid. The solution to be analysed is treated with silver nitrate and filtered. The precipitate is washed with weakly ammoniacal water, dissolved in dilute nitric acid, and then titrated with ammonium thiocyanate as in the determination of chlorides (Hesse 1921, and Raida 1923). The determination of cyanamid in body fluids or tissues is described on page 37.

*In this laboratory, calcium cyanamid did not yield a white precipitate with silver nitrate.

The action of cyanamid in the animal organism

Gergens and Baumann (1876) studied the physiological action of guanidin, dicyandiamidin and cyanamid in the animal body. Although each of these produced muscular disturbances such as spasms, the action of cyanamid alone will be described. Five mgm. of cyanamid injected in the lymph sac of frogs produced muscular fibrillation. Ten mgm. resulted in clonic spasms and opisthotonus, while 20 mgm. caused spasms and death.

Oral administration of 0.5 gm. to a rabbit was followed by clonic spasms of the muscles of the neck and the extremities. Death occurred after 12 hrs. Cyanamid could not be detected in the urine.* Since the weights of the animals were not given, the exact minimum lethal doses could not be determined.

The first thorough study of the action of cyanamid in rabbits, mice and pigeons was made by Coester (1896). In his introduction, he refers to the work of Lange who injected 0.05 gm. of cyanamid subcutaneously in a rabbit without any ill effect. In addition, a small dog which had received a similar dose, suffered an attack of vomiting and no other effects. A larger dog was given 0.1 gm. subcutaneously. After one hour it vomited copiously. Irregularity of the heart beat was also observed.

Coester in his own work used a synthetic cyanamid

*The writer was also unable to detect cyanamid in the urine of rabbits injected with it.

made from thio-urea. A series of animals of each species studied was injected subcutaneously with gradually increasing doses of cyanamid and the responses of the animals were then observed. The smallest dose that was lethal for one animal in the group was accepted as the minimum lethal dose.

Coester thus determined the minimum lethal dose in the series of twelve rabbits to be 0.35 gm. per kilo of body weight. The rabbits receiving lethal doses exhibited dilatation of the ear vessels, salivation, constriction of the pupils, fright, fall in body temperature, spasms, clonic movements of the extremities, weakness, diarrhea, and slowing of respiration, which became shallow and prolonged. Difficulty in walking or maintaining posture was also frequently observed. Reflex excitability was considerably heightened. Death occurred in over thirteen hours. Autopsy revealed hyperemia of the organs. Coester noted that the intensity of the paralysis increased with the size of the dose.

Mice receiving lethal doses of cyanamid presented a similar picture of increased irritability, paralysis, and spasms. The m. l. d. was 0.33 mgm. per gm. of body weight.

Pigeons injected with cyanamid likewise underwent the typical syndrome of muscular weakness, paralysis and disturbances in respiration. In addition, vomiting was noted, although Coester probably meant regurgitation. Coester

determined the m. l. d. for pigeons to be 0.32 gm. per 1000 gms. of body weight.

Transient paralysis was produced in rabbits, mice, and pigeons with one-third of the minimum lethal dose.

Coester concluded that cyanamid was primarily a toxic substance causing paralysis, and that the spasms observed were secondary phenomena.

As mentioned on page 1, Stritt studied the action of cyanamid and many of its derivatives. The following discussion is confined primarily to cyanamid. Dicyandiamid, Kalkstickstoff and Stickstoffkalk are described on page 14.

Stritt used cyanamid that was synthesized from potassium cyanate and calcium chloride or prepared from calcium cyanamid (Kalkstickstoff).

The cyanamid made from Kalkstickstoff was used either crude (unrecrystallized) or recrystallized from ether. The recrystallized cyanamid was used freshly prepared (less than a week old) for the experiments on frogs and rabbits. A few experiments were done on frogs with solutions that were seven days, and nine months old, respectively. One would not expect the seven-day or nine-months solutions to be as toxic as the freshly prepared one, since on long standing cyanamid slowly polymerizes into the less active dicyandiamid. In fact, for this reason only freshly prepared solutions were used in the majority of the experiments on rabbits. However, Stritt's experiments were too few in

number to establish any appreciable differences in toxicity of the freshly prepared, and older solutions as well as the crude and recrystallized preparations.

The following discussion will be limited mainly to the results obtained with the crude and recrystallized products used freshly prepared. The manner of determining the minimum lethal dose was identical with that of Coester.

By the injection of graduated doses in frogs, Stritt found that 10 mgm. per 50 gm. of body weight of the freshly prepared crude cyanamid caused spasms and death in one frog after 96 hours, while 20 mgm. of the synthetic product was lethal after 144 hours. Stritt's work does or does not confirm that of Gergens and Baumann, according to whether one considers the results obtained with the synthetic compound or crude cyanamid. More observations would have clarified this point.

In the following experiments with recrystallized cyanamid the concentration of the solution varied from one to 50 per cent. Subcutaneous administration of varying amounts of the recrystallized compound to a series of four rabbits caused death after eight hours in one receiving as little as 0.1 gm. per kilo of body weight.

In another series of five rabbits receiving the crude product, Stritt found that the subcutaneous injection of 0.24 gm. per kilo of body weight was sufficient to cause death in one of the rabbits.

In general, symptoms of poisoning in these experiments

closely resembled those described by Coester (1896) and Hesse (1921), with the additional observation of tracheitis which was in agreement with Koelsch (1916,2).

The following experiment of Stritt is cited to show the rapid progression of symptoms after intravenous injection. Observations of blood pressure, pulse rate, respiratory volume and rate were recorded on a rabbit receiving 0.64 gm. per kilo of recrystallized cyanamid. The respiratory rate became slow and irregular and the volume of respiration fell steadily. The carotid blood pressure and the pulse rate declined gradually. Respiration ceased and reflexes were abolished before cardiac arrest occurred. The rectal temperature fell 5.0 degrees. No cyanamid was demonstrable in the urine which was free from sugar and protein. Death occurred in one hour.

Intravenous administration of 0.39 gm. per kilo of the recrystallized product caused death in one rabbit in 48 hours. Yet Stritt found that 0.1 gm. per kilo of the same preparation when injected subcutaneously was sufficient to kill one rabbit in eight hours. This discrepancy was probably due to the limited number of observations.

The oral dosage for the recrystallized preparation was much larger than the subcutaneous, e.g. 0.75 gm. per kilo of body weight proved to be the lethal dose for one out of five rabbits.

Stutzer and Söll (1910) fed cyanamid (pill form) to

several guinea pigs in an effort to determine its toxicity. They found 0.1 to 0.2 gm. per kilo had no effect, whereas 0.4 gm. per kilo was followed by slowing of the heart, spasms and death in 30 hours.

The next investigation in chronological order is that of Koelsch (1914;1916,2). The cyanamid used was described as grey-white, and of 97% purity.

Two-hundredths of a gm. per kilo administered by stomach tube to rabbits had no effect, while one gm. per kilo caused death in over 10 hours. The symptoms of poisoning were similar to those observed by Coester (1896), Stritt (1909) and Hesse (1921) after subcutaneous injection of cyanamid. In Koelsch's experiments the animals became restless, their heart rates and respiration were accelerated, and they exhibited increased peristalsis with defecation. Reflexes were difficult to elicit, and as the effects of the cyanamid increased, the respiration became shallow and difficult, the pulse faster and weaker, shivering and convulsive shaking of the body set in, and finally death occurred.

Autopsy revealed hemorrhages and foamy secretion in the trachea, hyperemia of the lungs, and watery secretion in the large and small intestines which contained few formed feces. This agrees substantially with the findings of Stritt (1909) and Hesse (1921) and amplifies the work of Coester (1896). Koelsch was of the opinion that cyanamid primarily disturbed

respiration and secondarily affected the muscular system.

Hesse (1921) using a recrystallized preparation made by Baum's method (1910), found in frogs that 50 mgm. of cyanamid per 50 gm. of body weight brought about typical poisoning symptoms in fifteen to thirty minutes. The minimum lethal dose was determined to be 20 mgm. although the number of animals studied was not given. In addition to observing spasms, he made the interesting finding that cyanamid alternately increased and decreased systolic filling of the heart in situ and caused diastolic pauses that were longer and shorter than normal. The heart finally stopped in systole. Records of the isolated heart furnished similar results. Bigeminy occurred frequently. Adrenalin injected intraperitoneally, abolished the diastolic pauses and prevented the slowing of the heart. Inasmuch as the heart was beating vigorously, the phenomena could not be due to paralysis of the myocardium. Atropine had no effect, so the irregularities in heart action were probably not of vagal origin. Since caffeine did not prevent the influence of cyanamid, Hesse concluded that the action of the drug was due to stimulation of the "excitomotorische automatische Nervenelemente". In frogs injected with cyanamid the second Stannius ligature was "unwirksam", although Hesse did not state exactly what occurred when the second ligature was applied. He concluded, however, that cyanamid was responsible for "Schädigung der intraventricularen automatischen nervösen Apparate".

Subcutaneous injections of 0.3 to 0.4 gm. of cyanamid per kilo in rabbits caused death in 12 hours. The number of animals observed was not mentioned. The picture of poisoning was similar to that described by the other investigators, although the occurrence of spasms was not recorded. Pulse records were taken following intravenous injections in rabbits of 0.4 gm. of cyanamid per kilo, given in divided doses. Cardiac irregularities similar to those noted in frogs were observed. In addition, plateau formation occurred which Hesse points out is analogous to the bigeminy noted in the frogs. At autopsy the vessels of the trachea and the small intestine were injected.

Although the injection of 0.96 gm. of cyanamid in three portions over a period of several days in a rabbit weighing 1.9 kilos, resulted in diarrhea and 15 per cent loss of weight, Hesse stated that chronic administration of cyanamid had no effect and that the diarrhea was not caused by the drug. At death, however, the small and large intestines were hyperemic and the kidneys were enlarged. In a second experiment a total dosage of 1.0 gm. injected over a period of 14 days in a rabbit weighing 2.2 kilos caused a 22 per cent loss of weight.

The injections of 0.04 gm. of cyanamid per kilo into dogs and cats brought about paresis of the extremities, a fall in body temperature, coarse tremors, vomiting, and diarrhea. The animals recovered Hesse 1921). This increased toxicity for dogs is in agreement with the work of

Stritt (1909).

A dose of 0.08 gm. per kilo in cats caused death in 14 hours (Hesse 1921).

Horn (1933) in two dogs determined the average minimum lethal dose of cyanamid injected subcutaneously to be 0.10 gm. per kilo. The animals died a week after the injection.

The minimum lethal doses reported by the different authors (Table I) vary mainly because of the insufficient number of observations, and partly because of the purity of the cyanamid used. The purity of the cyanamid is an important factor in the determination of the exact minimum lethal dose, but not of the relative minimum lethal doses as established by most of the investigators.

The action of derivatives of cyanamid

Under this heading will be discussed dicyandiamid, Kalkstickstoff, Stickstoffkalk, and various other derivatives of cyanamid.

The injection of 100 mgm. of dicyandiamid per 50 gm. of body weight caused death without convulsions in frogs. A dose of 0.29 gm. of dicyandiamid per kilo subcutaneously injected was the smallest lethal dose in a series of four rabbits. The symptoms were weakness and tracheitis. The rabbit died after 29 hours (Stritt 1909).

Hesse (1921) determined that dicyandiamid was one-third as toxic as cyanamid.

Table 1

Minimum Lethal Doses Of Cyanamid in Mammals.

Author	Animal	Cyanamid in gm. per kilo	Route of administration
Coester	rabbit	0.35	subcutaneous
Stritt	"	0.10	"
Hesse	"	0.30 to 0.40	"
Stritt	"	0.39	intravenous
Hesse	"	0.40	"
Stritt	"	0.75	oral
Gergens and Baumann	"	0.50 total dose	"
Koelsch	"	1.00	"
Horn	dog	0.10	subcutaneous
Hesse	cat	0.08	"
Stutzer and Söll	guinea pig	0.40	oral
Coester	mouse	0.33	subcutaneous

Löw (1908) observed no ill effects after the injection of two mgm. of dicyandiamid per gm. into a mouse.

According to Stritt, injections of Stickstoffkalk or Kalkstickstoff in rabbits were of approximately the same toxicity as dicyandiamid. Lethal doses of Stickstoffkalk produced weakness and tracheitis; lethal doses of Kalkstickstoff caused weakness, convulsions and tracheitis. The oral m.l.d's. for Stickstoffkalk and Kalkstickstoff were found to be 1.1 and 1.4 gm. per kilo respectively. Stickstoffkalk in a dosage of 0.025 to 0.05 gm. per kilo, i.e., 1/30 to 1/15 of the m.l.d., administered orally to rabbits daily for several days did not produce any signs of poisoning. The rabbits appeared normal and gained weight. Oral administration in rabbits of 0.15 gm. of Kalkstickstoff per kilo (1/10 of the lethal dose) for 20 days, or 0.30 gm. per kilo (1/5 of the lethal dose) for 10 days were likewise ineffective (Stritt 1909).

The daily feeding of calcium cyanamid in doses of 0.5 to 1.0 gm. to rabbits for 32 or 23 days respectively did not produce any noticeable changes (Koelsch 1916,2).

A dachshund weighing 8.8 kilo consumed less than 10 gm. of Kalkstickstoff, and vomited. No other effects were experienced. He was then given one gm. by stomach tube, and developed signs of weakness which disappeared after several days. Three weeks later he received 0.23 gm. orally. Vomiting did not occur, but the animal gradually became weaker and died in

16 hrs. At autopsy, the intestines contained fluid feces (Stritt 1909).

Hesse (1922) did several experiments on certain derivatives of cyanamid. Injections of liquid dimethylcyanamid in rabbits in doses of 0.1 gm. per kilo caused a fall in body temperature followed by death in twenty-four hours.

One-tenth of a gm. of liquid diethylcyanamid per kilo produced toxic symptoms in rabbits while 0.2 gm. per kilo was lethal. The animals exhibited slowing of the respiration, paresis, fall in temperature, narcosis and injection of the trachea. Diethylcyanamid like cyanamid proved to be more toxic for dogs than rabbits, a dose of 0.07 gm. being lethal.

Solid phenylmethylcyanamid, in a dose of 0.4 gm. by mouth, did not affect the temperature, pulse, and respiration, but caused shivering and chattering of the teeth. Death occurred in five hours.

In general, the administration of the various derivatives of cyanamid produce the same toxic phenomena as cyanamid itself. In some cases, however, the progress of the poisoning is much slower. This is probably due to the time of conversion of these substances into cyanamid.

Animal experiments on the administration of cyanamid with other drugs

A. The administration of cyanamid with alcohol

If cyanamid and alcohol are taken internally, some of the actions of each are intensified and prolonged. The clinical picture is described in detail on pages 27 to 28. The administration of 1.5 gm. of cyanamid per kilo by stomach tube, followed 15 minutes later by one gm. of 96 per cent alcohol per kilo to one rabbit produced air hunger, whereas this dose of cyanamid alone did not have the same effect on another rabbit. Both, however, soon showed signs of cyanamid poisoning and died in two hours. The dose of 1.5 gm. of cyanamid used is by no means a small one as Koelsch says, for one gm. per kilo is the minimum lethal dose (Koelsch 1916,2).

In another experiment Koelsch first administered 0.2 gm. of cyanamid per kilo, and then a total dose of 3 gm. of 96 per cent alcohol orally. Hyperemia of the head and ears was observed in the animal receiving both drugs but not in another animal receiving the same dose of cyanamid alone. Administration of the cyanamid and alcohol subcutaneously did not produce these results.

Measurements of blood pressure from the peripheral and central ends of the carotid artery in three animals receiving approximately both 0.5 gm. of cyanamid per kilo and 0.4 to 0.8 gm. of alcohol per kilo indicated that the fall in blood pressure is in the cephalic region.

A rabbit weighing 1.4 kilo was given 5 gm. of 96 per cent alcohol orally and injected intravenously with doses of 0.1 gm. of cyanamid until a total of 0.4 gm. was administered. The typical signs of cyanamid poisoning appeared by the time 0.3 gm. had been given, except that the fall in blood pressure was greater than with cyanamid alone.

The body temperature of animals injected with 0.1 gm. of cyanamid per kilo did not change appreciably. However, if one gm. of alcohol per kilo was administered with 0.1 gm. of cyanamid per kilo the temperature fell as much as four or even seven degrees. Hesse did not observe the constant hyperemia of the cephalic region that Koelsch reported.

The fall in temperature following administration of small doses of methyl alcohol in rabbits was also increased by the administration of 0.2 gm. of cyanamid per kilo.

The central nervous system of rabbits receiving one gm. of 96 per cent ethyl or methyl alcohol per kilo, plus 0.2 gm. of cyanamid per kilo, contained higher concentrations of alcohol than animals receiving alcohol alone.

The animals were killed when the body temperature had fallen to a sufficiently low figure. The values of alcohol in one case rose from 0.12 per cent in a control to 0.21 per cent in the experimental animal.

Apparently alcohol intensifies the fall in body tempera-

ture produced by cyanamid while the latter favors the accumulation of alcohol in the tissues. The great fall in blood pressure observed after the administration of alcohol and cyanamid is probably a combination of the effects of each.

The problem of how alcohol and cyanamid synergize the effect of each other is as yet unsolved. According to Koelsch, alcohol sensitizes the organism to cyanamid.

Monaco and Frattali (1918) believe that alcohol increases the solubility of the CN and NH_2 groups in the body. They state, "Tute queste serie di esperienze che dovreblero esser completate con altre allo scopo di vedere se i disturbi soferti dagli operai siano d'indole vasomotoria, monstrano che la tossicità della calcio-cianamide è dovuta al gruppo CN rinforzato dal gruppo NH_2 che spesso si manifesta dotato di intensa azione biologica in mottissime sostanzi derivate.... Si deve pure ammettere tanto mell'uno che nell'altro caso chè l'alcool o aumenta la solubilità di questa sostanza nell' Organismo, oppure esalta, rendendola manifesta, l'azione di essa specialmente reguardo all'influenza vasale".

B. <u>The administration of cyanamid with other drugs</u>

Cyanamid in small amounts seems either to have the property of activating subthreshold doses of certain drugs or of intensifying the effects of small doses. On the other hand, many drugs augment the temperature-lowering effect of cyanamid.

Hesse (1922) found that 5 to 7 mgm. of cyanamid in frogs increased the convulsive effect of 0.005 mgm. of strychnine or 0.1 mgm. of picrotoxin.

On the other hand, cyanamid, in frogs, did not activate extremely small doses of yohimbin, cocaine, curare, nicotine, quinine sulphate, caffeine sodio-salicylate, and strophanthin.

Injections of 0.1 gm. of cyanamid per kilo in rabbits produced no significant changes in body temperature. However, when in addition, Ringer solution saturated with chloroform was injected, a fall in body temperature of 1.8 to 2.5 degrees occurred in 45 to 60 minutes (Hesse 1922).

Dittrich (1924) demonstrated that the injection of subthreshold doses of cincophen (0.25 gm. per kilo) and 0.1 gm. of cyanamid (subcutaneously) or 0.15 gm. (orally), induced a greater fall in body temperature, than the same dose of cincophen alone. The greater the time interval between the administration of cyanamid and cincophen, the greater was the fall in body temperature. A fall of 12 degrees was observed in one case where cyanamid was given one hour before the cincophen.

The administration in rabbits of 0.25 gm. of urethane per kilo together with 0.1 gm. of cyanamid per kilo did not appreciably lower body temperature, whereas twice that dose of urethane plus cyanamid did cause a drop in body temperature (Hesse 1921).

Sodium veronal, in doses of 0.05 gm. per kilo, and

sodium nitrite, respectively, resulted in a fall in temperature in rabbits when administered with 0.1 gm. of cyanamid per kilo. Yohimbin, or chloral hydrate in small doses, produced a drop in body temperature in rabbits when administered with 0.2 gm. of cyanamid per kilo (Hesse 1921).

Although cyanamid in concentration of 0.7 to 14 per cent had no effect on intestinal strips, it did render active subthreshold doses of papaverine, codeine, morphine, and atropine (Hesse 1921). It did not augment the action of physostigmine and pilocarpine.

According to Janusche (1913) sodium bromide in doses of 0.7 to 1.0 gm. per 200-250 gm. guinea pigs, did not result in narcosis, whereas this amount and even smaller amounts when given with 0.1 gm. of cyanamid caused narcosis in Hesse's experiments.

Determination of bromine in the central nervous system of guinea pigs were performed after the administration of 0.3 to 0.4 gm. of sodium bromide and 33 mgm. of cyanamid. There was a rise in the concentration of bromine from 0.09 to 0.13 per cent in one of these experiments (Hesse 1922).

An intensification of the diuretic action of 0.03 gm. of theobromine sodio-salicylate was observed with 0.1 gm. of cyanamid (Hesse 1921).

Increases in theobromine excretion were observed in animals given theobromine sodio-salicylate together with 0.2 gm. of cyanamid (Hesse 1921).

The administration of 0.1 gm. of cyanamid prevented glycosuria that otherwise would have occurred following the administration of glucose (Raida 1923).

After determining the threshold stimulus of the vagus in a rabbit, Hesse (1921) injected 50 mgm. of cyanamid and found that the nerve was still responsive to an electrical stimulus. In addition, however, he noted that atropine no longer paralysed the vagus. Apparently cyanamid inhibited the action of atropine. Cyanamid, however, did not abolish the antagonism of muscarine and atropine.

Cyanamid did not augment the inhibitory effect on respiration in a rabbit caused by the administration of 0.4 to 0.8 mgm. of morphine per kilo.

The convulsive action of camphor 0.1 gm. per kilo was not rendered effective by simultaneous administration of 0.1 gm. of cyanamid per kilo in rabbits (Hesse 1922).

The effect of phlorhizin in one per cent sodium carbonate was not increased by 0.2 gm. of cyanamid per kilo (Hesse 1921).

Amidopyrine when given with 0.2 gm. of cyanamid per kilo did not exert any greater antipyretic action in fever induced by bacterium coli than when administered alone.

Subcutaneous injections of cyanamid with sodium formate or sodium potassium tartrate yielded equivocal results when determinations of the amounts of unoxidized materials in the urine were made (Raida 1923).

The output of uric acid was not altered by the adminis-

tration of 0.2 gm. of cyanamid and 0.5 gm. of cincophen (Dittrich 1924).

Injections of cyanamid did not influence the conjugation of amylenehydrate to glycuronic acid as indic ated by the absence of changes in rotation, negative reduction and fermentation of the urine. It did, however, increase the formation of glycuronic acid from phenol and chloral hydrate. Determinations of chloral hydrate and chloroform gave negative results (Hesse 1922).

The action of cyanamid in the human body

Two types of reaction will be discussed. The first deals with the local and general effects of calcium cyanamid. The second is concerned with the internal administration of calcium cyanamid and alcohol.

1. Local and general effects of calcium cyanamid

(a) Local effect of calcium cyanamid

Koelsch (1916) was one of the first to describe the dermal and mucosal lesions resulting from contact with calcium cyanamid.

The exposure occurs in the manufacture and packing of calcium cyanamid or in spreading it as fertilizer in the fields. The lesions are ulcerative, inflammatory, exudative, and slow healing. They occur on the skin, and mucosal surfaces of the upper respiratory tract.

Monaco and Frattali (1918), Van Husen (1919), Schoofs (1933), Van Itallie and Bylsma (1928), Hope, Hanna, and Stallybrass (1923) also have reported on similar skin injuries following exposure to calcium cyanamid. Laurentier (1925) has described an interesting case of generalized herpes zoster due to contact with calcium cyanamid.

(b) General effects of calcium cyanamid

Linneberg (1933) published a report on a case of calcium cyanamid poisoning in which the symptoms consisted of headache, dysphea, almond-odor on breath, edema and erythema of face, neck and hands. There is no mention of the consumption of alcohol in this case despite the resemblance to the alcohol-cyanamid syndrome.

Ohnsorge (1928) reported on two cases of exposure to calcium cyanamid where the symptoms were localized myelitis and polyneuritis in one case, and Landry's paralyis in the other.

At the same time Mann (1928) described a case of calcium cyanamid poisoning in which slowly increasing paralysis of the arms and legs began to develop two weeks

following contact with calcium cyanamid used as a fertilizer.

The writer with Henstell and Himwich (1934) reported on a case of peripheral neuritis in an individual who had been employed shovelling calcium cyanamid in an enclosed bin for three months previous to the onset of the illness. The patient had also drunk alcoholic beverages in moderate amounts for years, although his consumption had been limited for several months preceding his admission to the hospital.

Since Jolliffe and Colbert (1936) have pointed out that vitamin deficiency is the etiological agent responsible for the peripheral neuritis in alcohol addicts, the influence of alcohol per se can be excluded in this case. No other contributory factors were noted.

2. Administration of cyanamid with other drugs

The administration of cyanamid with another drug frequently results in enhancing the action of the latter drug (page 20), (Hesse 1921, 1922; and Dittrich 1924). In the case of alcohol and cyanamid, however, it would seem that the two drugs

synergize the action of one another (Koelsch 1914; 1916,2 and Hamilton 1925).

Koelsch (1914; 1916,2) was the first to describe the syndrome developing after contact with calcium cyanamid and alcohol. It occurred mostly in people who drank alcohol after exposure to calcium cyanamid occupationally or from inhalation of the powdered material while using it as fertilizer.

The symptoms of exposure to cyanamid and alcohol are mainly circulatory and respiratory in nature. The dorsal and ventral surfaces of the upper regions of the body are blue-red as in fever. The arms are lighter in color, but still hyperemic. The eyes are injected; the gums and earlobes are red; while the oral mucosa is pale. Light shivering occurs at times. Respiration is accelerated to 20 or 25 per minute and is deeper than usual. The respiration are thoracic and the inspirations frequently audible. Some coughing occurs as a result of hyperemia of the upper respiratory tract. The heart rate is increased to 100 or 130 per minute. The blood pressure may be normal or lowered. Spectroscopic examination reveals formation of methemoglobin and cyanhematin (Koelsch 1916). The sensorium and reflexes are unchanged. Dizziness and vomiting may occur. The attack lasts one or two hours depending upon the amount of alcohol

taken. Recovery occurs without any lasting effects. There is no cumulative effect despite two to three years occupational exposure.

Hesse (1921) narrates an interesting incident of an individual who took 150 mgm. of cyanamid by mouth with no apparent effect, and later in the same day had a glass of beer. A typical acute attack of headache, malaise, retching, exanthema of the head and neck was experienced without any permanent effects.

Hesse (1921) has reported on several clinical experiments where cyanamid and other drugs were taken. Moreover, 0.1 gm. of theacylon and 100 mgm. of cyanamid both orally taken did not result in increased diuresis. Doses of 50, 100, and 150 mgm. of cyanamid alone taken orally had no effect.

Theacylon, unlisted in "New and Non-Official Remedies", is probably a German proprietary containing one of the xanthine bases.

Mechanism of action of cyanamid

1. Effect on enzymatic activity

Dittrich (1924) made a study of the influence of cyanamid on fermentative reactions. He found it had no effect on the action of trypsin, pepsin, invertin, amygdalin, serum lipase and peroxidase. However, the activity of catalase was inhibited by cyanamid. This was measured by titrimetric and volumetric methods.

In the first method he added cyanamid to a mixture of blood, physiological saline and hydrogen peroxide, and at intervals titrated portions of the solution with potassium permanganate. Cyanamid in concentrations of 1:40,000 prevented the breakdown of 60 per cent of the added peroxide at the end of one-half hour. The injection, however, of 0.2 gm. of cyanamid per kilo into a rabbit had no effect on blood catalase.

Sodium cyanide (1:8,000) was found to prevent the decomposition of 70 per cent of the added peroxide at the end of one-half hour, while ammonium thiocyanate (1:4,000-1:40,000) inhibited the breakdown of 80 per cent. Nevertheless, Dittrich discounted the possibility of a chemical relation between cyanide and cyanamid. The degree of inhibition of catalase activity varied directly with the concentration of cyanide or cyanamid used.

In Dittrich's second method, an eudiometer tube was used to measure the volume of oxygen liberated from a mixture of blood, saline and peroxide, which had been treated with cyana-

mid. Almost quantitative transformation of the peroxide occurred in the absence of cyanamid, but in one case as much as 72 per cent was unchanged in the presence of 144 mgm. of cyanamid. Using Raida's method (1923) for the determination of cyanamid he was able to recover about 26 per cent of it. Since appreciable formation of urea did not occur, the cyanamid was apparently not converted into urea. The nature of the compound formed was not revealed.

Furthermore the ferrous sulphate catalysis of peroxide was also inhibited by cyanamid without significant formation of urea.

Cyanamid in a concentration of 1:230 did not inhibit fermentation in the liver. It did, however, augment the inhibitory effect of formalin or phenol on the oxidation of glucose which had been added to liver. The oxidation of the glucose was measured by the amount of carbon dioxide formed. Dittrich was of the opinion that the changes he observed were due to permeability phenomena in the liver cells.

2. Effect on oxidation—reduction

Dittrich next attempted to demonstrate an inhibition of the oxidation of benzol to phenol by cyanamid. This was not conclusively proved although a decreased formation of conjugated sulphates was observed.

Definite evidence that cyanamid hinders the reduction of m-dinitrobenzol to nitrophenylhydroxylamine was afforded by the following experiments. Colorimetric determinations of

nitrophenylhydroxylamine in frog tissue with cyanamid added in concentrations of 1:50 to 1:2,000 in frogs injected with 1.5 mgm. of cyanamid per gm., gave lower values than in controls without cyanamid. One leg of the animal served as control for the experiments where cyanamid was injected. These differences were about 50 per cent and of absolute value because standards of nitrophenylhydroxylamine were used. Controls receiving injections of potassium chloride to cause heart injury analogous to that which might be produced by cyanamid were negative.

Cyanamid in concentrations of 1:200 inhibited respiration of frog liver and muscle in the Warburg apparatus.

Dittrich concluded from this study that cyanamid inhibits oxidation and reduction in vitro and in vivo.

Glaubach (1926) attacked the problem of the point of action of cyanamid by performing determinations of glutathione in tissues of animals injected with cyanamid. Titration with iodine affords a method for the quantitative estimation of glutathione since reduced glutathione combines with iodine and cyanamid does not. After establishing normal values for glutathione in minced frog muscle, Glaubach injected 50 mgm. of cyanamid in 0.6 per cent saline and determined the glutathione content of the muscle at varying intervals. No change was observed at the start of the experiment but after four hours the concentration of glutathione in one instance fell from 0.144 per cent to 0.067 per cent. Controls were 0.118 at

at the beginning and 0.114 per cent at the end of the exper-ment.

Studies of the behavior of cystin in animals poisoned with cyanamid were next performed. Normally, cystin is transformed into cystein. Hydrogen cyanide favors while cyanamid opposes the transformation. Again, an iodine titration method was employed; one cubic centimeter of N/100 iodine being equal to 1.2 mgm. of cystein. Tissues were placed in phosphate buffer, with cyanamid and cystin. After they had stood for 8 or even 22 hours, the tissues were analyzed. In the presence of cyanamid the value of the iodine titration was considerably lower than in tissues not treated with cyanamid. This could be explained by the fact that cystin does not form cystein when cyanamid is present.

Controls with cyanamid and phosphate alone, or with cyanamid, phosphate, and cystin were negative. However, a control of the untreated tissue allowed to stand several hours was not carried out. Neither was any control run on the stability of the N/100 iodine for that period of time.

In addition to the iodine titration method, Glaubach measured the time in minutes for the decolorization ofa definite amount of N/100 iodine by frog tissue treated with cyanamid and cystein. He found that it was longer than in tissue containing cystin without cyanamid. This would possibly indicate that since cystein was not being formed it would take longer for the decolorization to occur than other-

wise. He did not rule out the action of glutathione in this set of experiments.

Similar experiments were performed on the frogs after the removal of one leg for the control. Glaubach says that they were then poisoned in vivo with cyanamid, but does not explicitly state how this was accomplished. The control leg muscle showed an increase in material titratable with iodine on standing whereas the poisoned muscles exhibited a fall. If cystin were added the values were also lower for the poisoned muscle.

The administration of cystin, cystein, oxidized glutathione and sodium thiosulphate did not antagonize cyanamid poisoning.* Glaubach gives no data how this was proved.

In a second paper, Glaubach (1926) reported on a study of cyanamid on cystein and cystin in vitro. A solution of cystein on standing at room temperature slowly loses its property of reaction with sodium nitroprusside. If cyanamid is added the nitroprusside reaction decreases much earlier. Glaubach was apparently unaware of the fact that cyanamid itself gives a positive nitroprusside reaction.

A solution of one per cent cystein did not react with appreciably less N/100 iodine after standing seven hours but a solution of cystein and cyanamid underwent a great diminution in its ability to reduce iodine. It would appear that cystein is converted into some other compound, possibly cystin.

*Hesse(1921) could not demonstrate a detoxification of cyanamid by glycin and sarcosin in frogs, dogs and rabbits.

If cyanamid is added, the pH of the solution changes from 5.5 at the beginning of the experiment to between 8.5 and 9.0. The significance of this observation lies in the fact that the optimal pH for the conversion of cystein to cystein to cystin is 7.5 to 8.0. However, no evidence of the formation of cystin was furnished by the determination of the optical rotation before and after the addition of cyanamid.

More sulphide can be detected in the cystein-cyanamid solution than in the cystein solution as determined by treatment of the solution with lead acetate and potassium hydroxide.

Kühnau (1927) confirmed and amplified Glaubach's work considerably. The passage of hydrogen through freshly drawn blood for an hour or more, split off sulphide from some substance in the blood, as demonstrated by darkening of paper impregnated with lead acetate. This sulphur yielding substance was detectable in the erythrocytes or protein-free blood filtrates, and was apparently identical with glutathione which is known to be present in blood. Both potassium cyanide and the dinitrile of malonic acid, when added to the blood or injected into cats or rabbits had the same effect, i.e., appreciably increasing the amount of obtainable sulphur. On the other hand, cyanamid under similar circumstances considerably reduced the amount of sulphur that was split off from the compound in the blood.

Hydrogen was found to produce a rise in the labile

sulphur arising from the reduction of cystin. Again, potassium cyanide augmented, while cyanamid lessened the amount of sulphur formed.

In all cases, the sulphur increase was accompanied by an increased nitroprusside reaction.

Quantitative determination of the blood sulphur and glutathione sulphur in several cases gave data essentially the same as that obtained by the qualitative procedures.

The action of cyanamid on the sulphide-containing compound may be compared with that of cyanide by the use of the following model suggested by Kühnau.

$$R_2S_2 + H_2 \rightleftharpoons 2R.SH$$

Cyanide forces the reaction to the right while cyanamid drives it to the left.

Moreover, this relation can also be expressed by the transformation of cyanamid to methylenediamine.

$$\underset{\text{cyanamid}}{NH{=}C^{\diagup\!\!\diagup NH}} + 4R.SH \rightarrow \underset{\text{methylenediamine}}{NH_2\text{-}CH_2\text{-}NH_2} + R_2S_2$$

Inasmuch as diamines hinder the conjugation of glycuronic acid it was thought that the possible conversion of cyanamid to a diamine and the action of the diamine thus formed would explain why cyanamid inhibits the conjugation of benzol with glycuronic acid (page 30).

3. Relation of cyanide and cyanamid

It has been suggested that cyanamid acts by virtue of

its transformation to cyanide. The following evidence indicates that this is not true.

1. Solutions of cyanamid after several weeks do not give a test for cyanide (Hesse 1921). *

2. Coester (1896) compared the effects of the injection of cyanide with those of cyanamid. He attributed the effects of cyanamid to a primary action of the latter rather than to cyanide derived from it. Coester was probably justified in this conclusion in view of the fact that cyanide produced true convulsions while the spasms observed in cyanamid poisoning were secondary to the respiratory disturbances. Moreover the salivation and diarrhea were characteristically produced by cyanamid. Koelsch (1916,2) and Stritt (1909) agreed with Coester that cyanamid per se was responsible for the effects following its administration.

3. The difference in the action of cyanide and cyanamid on the glutathione system has been pointed out on pages 34 and 35.

* This has been confirmed by the writer.

Isolation of cyanamid from tissues

Raida (1923) injected or added cyanamid to tissues and then determined the amount of cyanamid that could be recovered. After ether extraction of the tissue to which had been added 20 mgm. of cyanamid, a solution of ammoniacal silver nitrate was added. The silver cyanamid was filtered off and then washed with ammoniacal water until silver free. It was then dissolved in dilute nitric acid and titrated with 1/10 N ammonium thiocyanate.

9.45 cc of 1/10 N thiocyanate is to the 20 mgm. of cyanamid.

Citrated rabbit blood was treated with 20 mgm. of cyanamid and then the cyanamid determined as described above. The recovery was 96 per cent. Similar treatment of urine, liver-brain-and kidney-brei yielded 94 per cent recovery.

After thus demonstrating that he could recover added cyanamid with sufficient accuracy, Raida then injected 0.2 gm. of cyanamid per kilo intravenously into a rabbit and produced typical poisoning in about fifteen minutes. At this time carotid blood contained 0.45 mgm. of cyanamid per cc. Three-quarters of an hour later the carotid blood value of cyanamid was 0.24 mgm. per cc., and in four hours from this time there was no cyanamid detectable in the blood. The animal was killed and no cyanamid was found in the brain, liver, kidney, muscle or urine.

In a similar experiment in which a rabbit was injected

with 0.1 gm. of cyanamid per kilo the blood contained 0.24 mgm. of cyanamid per cc. after two hours. In this animal the administration of 1.0 gm. alcohol per kilo by stomach tube did not seem to affect the blood value of cyanamid, for as in the previous experiment no cyanamid was detectable in 5 cc. of blood four hours later.

The next animal received an emulsion of lecithin by vein, one hour preceding the cyanamid injection of 0.2 gm. per kilo. The typical symptoms of cyanamid poisoning appeared within an hour, but in contrast with the previous experiment the blood now contained 5.9 mgm. cyanamid per cc. An hour later the blood level was 2.8 mgm. per cc. The animal exhibited convulsions and died two and a quarter hours after the injection. At death the brain apparently contained 4.2 mgm. of cyanamid per 100 gm. and the muscle 1.7 mgm. per 100 gm. although Raida gives the impossible values of 21 mgm. per cent in 5 gm. of brain brei, etc. It would seem that lecithin slows the destruction of cyanamid in the organism.

Transformation of cyanamid in the organism

Raida (1923) injected 0.1gm. of cyanamid and 0.03 gm. of theobromine-sodio-salicylate per kilo to determine whether the former was eliminated in the ensuing diuresis but could not find any cyanamid in urine collected one, two, four, and six hours later. Moreover, dicyandiamid, guanidin, cyanide, and thiocyanate were not detectable either.

The fate of cyanamid added to various tissues was next

studied. Blood and liver of various animals were treated with cyanamid; chloroform or sodium fluoride added: and the suspensions incubated for several days. The urea and ammonia in the liver alone increased as a result of autolysis; there was no change in the concentration of creatinin. Guanidin and dicyandiamid were not found. No data are given on these experiments although they were controlled. Raida in another experiment incubated 20 cc. of liver brei and 80 mgm. of cyanamid for ten days. Analyses yielded the following.

35.2 mgm. of cyanamid
4.1 mgm. of ammonia, corresponding to 10.1 mgm. of cyanamid
8.3 mgm. of urea corresponding to 5.8 mgm. of cyanamid
No guanidin or dicyandiamid

Raida was thus able to account for 51.1 mgm. of cyanamid. Tests for cyanide, and thiocyanate were negative. The unrecovered cyanamid was probably transformed into an unknown product, although polymerization should not have occurred due to the acidity of the medium.

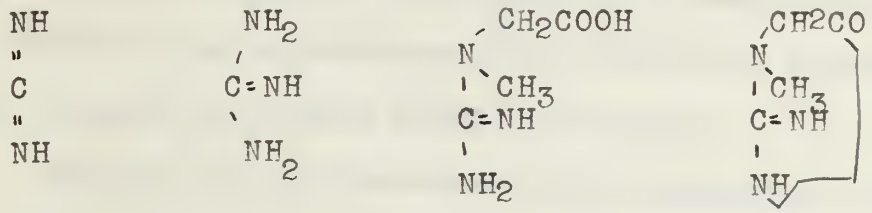
Horn (1933) was unable to isolate guanidin from the urine of rabbits which had been injected for ten days with a total of 10 gm. of cyanamid. Moreover, he could not demonstrate a relation between the changes in blood sugar and body temperature in animals injected with guanidin or cyanamid.

Relation of cyanamid to creatin

The relation of cyanamid to creatin has been known since Volhard (1868) combined cyanamid and sarcosin in alcohol to form creatin. Strecker (1870) and Rosengarten and Strecker (1871) were able to obtain better yields by allowing

aqueous solutions of sarcosin and cyanamid to stand at room temperature for several days in the presence of ammonia.

The chemical relation of cyanamid to guanidin, creatin, and creatinin is indicated by the following formulae.



cyanamid guanidin creatin creatinin

Before discussing the relation of cyanamid to creatin and creatinin in blood, the writer has found it necessary to comment on the status of the presence of creatinin and creatin in blood.

The nature of blood creatinin

Behre and Benedict in 1922 adduced evidence which they interpreted as indicating that significant amounts of creatinin were not present in the blood. In 1935 Behre and Benedict ,and in 1936 Benedict and Behre concluded that there was no preformed creatinin in blood. On the other hand, Danielson(1936) stated that most of the " chromogenic material in normal plasma is creatinine ". The data of Langley and Evans (1936) would tend to favor the second of these opinions.

Gaeblerand Keltch (1928) concluded that "all the chromogenic substance removed by Lloyd's reagent and released again was identified as creatinine, both in normal and retention blood.* Creatinine is present in large amounts in blood during experimental and nephritic retention and is also present in normal blood of cattle".

Gaebler in 1930 abandoned the conclusion that creatinin was present in blood. He now maintained that the "blood of normal dogs contains a substance, other than creatine, yielding creatinine in isolation experiments. A similar substance is present in human blood". When renal function is impaired this substance accumulates. That is, he now believed that creatinin was present as a result of adsorption and release from Lloyd's reagent. Gaebler (1937) has recently concluded that "a part of the apparent creatinine of normal serum ultrafiltrates simulates creatinine in its precipitation behavior, is not identical with it, but may be related to it".

From this state of affairs it would seem that creatinin is or is not present in blood according to one's interpretation of the experimental data.

There are several factors responsible for these discrepancies. It is possible to reconcile some of them by a brief review of some of the data. Much of the evidence that has been presented is indirect, and many of the conclusions thus derived even more so. The situation is simplified from the

*Lloyd's reagent is a hydrated aluminum silicate.

start by excluding the results obtained with heat coagulation filtrates, since they yield high values (Benedict and Behre 1922). Moreover, analyses on unlaked filtrates have also been omitted because most of the work was done on laked filtrates.

Behre and Benedict (1922) demonstrated the following.

1. Sodium carbonate did not produce any appreciable color when used as the source of alkali in the Jaffé reaction, with amounts of creatinin similar to those present in blood filtrates. However, considerable color developed if it were employed in the colorimetric determination of creatinin in picric acid blood filtrates.

2. Hot alkali destroyed the chromogenic property of pure creatinin but not of tungstic or picric acid blood filtrates of beef blood.

3. Kaolin quantitatively removed creatinin from pure solution or added to picric or tungstic acid filtrates, of beef, dog or human blood, as determined by analyses of the filtrates before and after kaolin treatment. However, it did not adsorb the chromogenic substance of picric acid filtrates, determined also by differences. Gaebler (1930) confirmed these results on picric acid filtrates.

Behre and Benedict (1922) concluded that creatinin was not present in blood in detectable quantities, simply on the basis of the dissimilarities of pure creatinin and the

chromogenic substance of blood filtrates, in the respects mentioned.

Danielson (1936) repeated and amplified several of these experiments and obtained the following results.

1. No difference was demonstrable between the color development in pure creatinin and ultra-blood filtrates. Unfortunately he did not work with picric acid filtrates, and employed larger amounts of creatinin than Behre and Benedict so that his experiments were not analogous with theirs.

2. Hot alkali destroyed most of the chromogenic property of pure creatinin, ultrafiltrates or tungstic acid filtrates. This is in agreement with the results on tungstic or picric acid filtrates (Behre and Benedict 1922). However, Danielson did not consider this as evidence that the chromogenic substance was not creatinin. In fact he cited proof that other chromogenic substances were formed by treatment of filtrates with sodium hydroxide. His evidence for this was that kaolin removed from filtrates a chromogenic substance which behaved like creatin. Blood filtrates treated with kaolin to remove this material and then heated with sodium hydroxide still gave similar colorimetric values, although the colors were tinged with brown. Boiling with alkali following the omission of kaolin treatment yielded the same values as before. Since kaolin removed the "creatinin", then other chromogenic substances must have been

formed in the reaction with sodium hydroxide. Lloyd's reagent was as effective as kaolin. Unfortunately, Danielson did not repeat these experiments on picric acid blood filtrates as Behre and Benedict had done. It is conceivable he would have obtained the same results as with the tungstic acid filtrates, since he confirmed the observations of Behre and Benedict on tungstic acid filtrates.

3. Kaolin quantitatively removed 68 to 94 per cent of the "creatinin" of ultrafiltrates or tungstic acid filtrates, as determined by colorimetric analysis of the material eluted from kaolin by the use of magnesium oxide. It will be recalled that in 1922 Behre and Benedict were unable to remove any of the chromogenic substance of blood filtrates, according to the values obtained on filtrates after shaking them with kaolin. At this time they probably did not analyse the material adsorbed by kaolin, or they might have obtained the same results as Danielson. Since then Benedict has done so (page 45). Pure creatinin or the "creatin" that was recovered from the kaolin was inactivated by hot alkali (Danielson 1936). After kaolin treatment approximately 40 per cent of the "creatinin" originally present (determined colorimetrically in the usual way) remained behind in the laked filtrates, but 10 per cent in the ultrafiltrates. Inasmuch as kaolin only removed, on the average, 87 per cent creatinin from solutions of pure creatinin and but 73 or 80 per cent from the laked filtrate and ultrafiltrate, respectively, these recoveries are not as low as they might have

seemed.

Danielson thought it likely that the picric acid used by Behre and Benedict in preparing their filtrates "hindered the adsorption of creatinine or during the process of protein precipitation a new chromogenic material is formed which is not adsorbed by kaolin". This might well have been the case, although Danielson neglected to repeat the same experiments on picric acid blood filtrates. According to Gaebler (1937), Benedict in 1930 eluted chromogenic substance from kaolin after shaking picric acid filtrates with it.

Danielson concluded that there was considerable creatinin in blood plasma, judging from the similarity of the chromogenic substance of blood filtrates and pure creatinin. The points of similarity were the adsorption on kaolin and the destruction by hot alkali of the material eluted from the kaolin.

Gaebler (1930) carried Danielson's experiment with hot alkali one important step further. He found that Lloyd's reagent removed only traces of chromogenic substance from tungstic and filtrates that had first been treated with hot alkali.

The use of kaolin or Lloyd's reagent to adsorb chromogenic substances from blood filtrates and the subsequent recovery of "creatinin" from Lloyd's by treatment with alkali, has been reported by many investigators (Gaebler

and Keltch 1928; Gaebler 1930; Hayman, Johnston, and Bender 1935; Langley and Evans 1936). Benedict and Behre (1936) were unable to obtain positive results in a single experiment. The possible reasons for their findings will be discussed later.

Gaebler and Keltch (1928) were able to isolate approximately 0.4 mgm. per cent of creatinin from normal ox blood by the following procedure. Following precipitation of the proteins by the aid of picric and phosphotungstic acids, the filtrates were treated with Lloyd's reagent. Elution of the chromogenic material from Lloyd's reagent was accomplished by the use of lead oxide. Unfortunately, the writers made corrections for errors of isolation, and reduced this figure to 0.09 mgm. per cent.

The blood of nephrectomized dogs or patients dying from nephritis yielded from 2.9 to 14 mgm. per cent of creatinin (analysis of the material eluted from kaolin). Samples of the same blood estimated colorimetrically before kaolin treatment revealed from eight to 20 mgm. per cent of creatinin.

From 4.4 to 10.6 mgm. per cent of creatinin was isolated as the double picrate. Why were higher values obtained by isolation than by kaolin adsorption, if kaolin quantitatively adsorbs creatinin? There are two possible explanations. Firstly, something may have interfered with the colorimetric determination before or after the treatment with kaolin, or

at both times. Substances that inhibit color development have been demonstrated in blood and muscle by Folin and Denis (1912). Secondly, there are chromogenic substances in blood in addition to creatinin. Hayman, Johnston and Bender (1935), among others, have supported the contention that blood contained several chromogenic substances as well as "creatinin".

Gaebler (1930) abandoned the conclusion earlier reached that the creatinin or creatinin derivative detected in normal blood is "accounted for by errors in detecting such small quantities by difference in the presence of a large amount of other chromogenic substances". He now expressed the opinion that the tungstic acid filtrates of normal blood contain 0.44 to 0.66 mgm. per cent of creatinin; in retention the values are increased. Picric acid filtrates gave parallel but smaller values.

"Creatinin" was determined by adsorbing the filtrate on Lloyd's reagent and then recovering the chromogenic substance by elution with magnesium oxide. Lead oxide was also suitable for elution as shown previously. The material eluted was estimated colorimetrically as creatinin. It could also be quantitatively precipitated as creatinin potassium picrate and converted to creatinin zinc chloride. The nitrogen analysis on the final product agreed with the theoretical value for creatinin. In agreement with Behre and Benedict (1922), Gaebler (1930) found that kaolin treatment did not

alter the chromogenic reaction of picric acid filtrates. Moreover, he showed that kaolin adsorbed all the "creatinin" from picric or tungstic acid filtrates, as indicated by the insignificant values obtained with the use of Lloyd's reagent after preliminary kaolin treatment.

Since Gaebler was unable to detect any difference in the picric acid filtrates (1930), or in laked blood ultrafiltrates (1937), as determined colorimetrically before and after kaolin treatment, he concluded that creatinin was not present. He neglected the possibility that there may be something present in blood which is chromogenic and not creatinin (although in 1930 he admitted the existence of other chromogenic substances in blood), while at the same time the chromogenic effect of the creatinin present is obscured or inhibited. He pointed out the important fact that after kaolin treatment he was able to elute chromogenic material from the kaolin but would not recover any more chromogenic substance from the filtrate by the use of Lloyd's reagent. This would indicate that creatinin had been present and was removed, for there was no doubt that the final product was creatinin, as he had shown in 1930. Further possibility that there may be other chromogenic substances present in blood filtrates, is supplied by the experiments that added creatinin is easily recoverable by kaolin treatment of laked ultrafiltrates, and yet the apparent creatinin value of the filtrate remains the same (Gaebler 1937).

After treatment with hot alkali, tungstic acid filtrates do not yield any appreciable amounts of creatinin according to determinations on the material eluted from Lloyd's (Gaebler 1930). Creatinin similarly is destroyed by boiling with alkali. This indicates the similarity of the two, but of course not their identity. This chromogenic substance after removal from Lloyd's can be isolated as creatinin zinc chloride and indubitably, identified as creatinin. On page 409 of his 1937 paper, Gaebler says that "this substance appears to become chromogenic in the process of adsorption and elution". The possible reason for the reaction with Lloyd's reagent is based on the fact that Lloyd's gives a strong oxidase reaction. However, on page 410, he states that "it has never been implied that the huge amount of chromogenic substance eluted from Lloyd's reagent in such cases" (referring to retention bloods) "originated in the process of adsorption and elution". The inference here is that this applies to normal bloods, although from his opinion expressed elsewhere, one is led to believe that the only difference between normal bloods and the retention bloods is the amount of the chromogenic substance present. So we have to assume that the retraction of this statement applies to both types of bloods.

Even if the treatment with Lloyd's does change the material into a chromogenic substance identical with and indistinguishable from creatinin, it would still be possible

to establish the presence of creatinin in blood, if it could be directly precipitated from it. This Gaebler seems to have done. In 1937 he reported the successful precipitation of approximately 60 per cent of the apparent creatinin as a picrate from the cellophane ultrafiltrates of the normal sera of various species, and in particular that of the ox. Precipitation was aided by the use of rubidium chloride and frequently also by potassium chloride. Although he has "considerable evidence that the chromogenicity of our precipitate is due to creatinin", Gaebler hesitates to conclude that it is creatinin until he has been able to precipitate it as the pure double picrate and thus finally identify this substance with creatinin.

Certain difficulties have been encountered in the precipitation of creatinin from blood. Pure creatinin added to collodion ultrafiltrates of dog blood was precipitated as the picrate in four or five days, while the apparent creatinin originally present did not precipitate at all in as many as fifteen days (Gaebler 1937). Behre and Benedict (1922) similarly could not precipitate creatinin, as creatinin zinc chloride, from a filtrate of dog retention blood, working with the chromogenic material they had adsorbed and then eluted from Lloyd's reagent. On the other hand, the slowness of precipitation, experienced by Gaebler, at least in the case of the dog, may in part be due to the fact that only small amounts of chromogenic substance are present. The

addition of pure creatinin in some cases seemed to increase the amount of chromogenic precipitate obtained. Moreover, creatinin of retention sera precipitated as rapidly as added creatinin, so again the amount present is apparently of importance. The action of possible inhibitory substances could also be a factor delaying the precipitation.

In Gaebler's work the assumption has been made that the acidity due to the picric acid added is not sufficient to "convert a non-chromogenic creatine-like substance to a precipitable chromogenic one". It is difficult to rule out this assumption if the precipitation can be accomplished only with the aid of picric acid.

Benedict and Behre (1936) applied the color reaction of creatinin with dinitrobenzoic acid to the quantitative determination of creatinin.* Excellent results were obtained with pure creatinin or the creatinin of urine, but not with blood filtrates. The difficulty experienced could possibly be explained by the presence of inhibitory substances which may or may not be chromogenic. The authors reported that rapid fading occurred, and the matching of colors was unsatisfactory. The application of this method to the material removed from a tungsto-molybdate filtrate by Lloyd's reagent yielded negative results. Benedict and Behre therefore concluded, on the basis of the dissimilarity of blood creatinin and pure creatinin according to this new color reaction that there was no preformed creatinin in blood.

*This color reaction has also been used for the determination of blood creatin on the material eluted from Lloyd's (Andes 1937).

However, Langley and Evans (1936) successfully determined blood creatinin by the above method. They used higher concentrations of alkali and dinitrobenzoic acid. The most interesting part of their work, however, was their successful use of Lloyd's reagent. The material adsorbed by and eluted from Lloyd's reagent, after contact with tungstic acid filtrates, behaved just like creatinin in the color reaction with dinitrobenzoic acid. The only noticeable difference in their use of Lloyd's reagent as compared with Benedict's was the use of magnesium oxide for elution, rather than carbonate which was employed by Benedict and Behre (1936). One is thus tempted to relate the failure of Benedict to demonstrate "creatinin" in this experiment to the method or chemical used. This is in contrast also with the many other individuals who have been able to adsorb "creatinin" from blood filtrates by the use of Lloyd's reagent (page 46). Interestingly enough, Behre and Benedict (1922) treated a filtrate of retention blood (dog) with Lloyd's reagent and were able to elute "creatinin" from the material adsorbed on Lloyd's. Hence these experiments of Behre and Benedict have little bearing on the existence of creatinin in blood.

Behre and Benedict (1935) maintained that creatinin is not present in blood, since "there remains even a single method by which it is possible to differentiate sharply between them when both (i.e., the two similar compounds) "are present in one solution". The single method he refers to is the failure of kaolin to change the chromogenic

properties of picric acid filtrates. In view of all the evidence presented to indicate the similarity of behavior of the chromogenic substance of blood filtrates and creatinin, and the weakness of the single point of differentiation, it is felt by the writer that adequate proof of the absence of preformed creatinin from the blood is lacking. Certainly, much more data must be presented to prove its absence from blood, since the weight of the evidence indicates its presence. The isolation experiments of Gaebler (1930) and the precipitation data of Gaebler (1937) afford strong evidence that part of the chromogenic substance in blood is true creatinin. The only disputable point as the writer sees it is the presence of creatinin in circulating blood.

The nature of blood creatin

The problem of the existence of creatin in the blood has been satisfactorily settled according to Behre and Benedict (1922). A specimen of blood obtained from a dog 46 hours after the ligation of both ureters, contained 12 mgm. per cent of preformed creatinin and 16.8 mgm. per cent of creatin as determined on heat-coagulated filtrates. Here Behre and Benedict with the use of Lloyd's reagent removed the "preformed creatinin" before acid hydrolysis of the filtrate. After hydrolysis of the filtrate which had been treated with Lloyd's reagent, a second treatment with Lloyd's extracted from it a chromogenic substance, 80 per cent of which was convertible to creatinin zinc chloride and was therefore creatinin.

They, therefore, concluded that creatin was normally present in blood, and that it was increased in nephritis.

Gaebler's criticism that the treatment with Lloyd's reagent may have been responsible for the formation of creatinin may be applied here to the question of creatin. According to his reasoning, creatin is not present in blood either, since it is not demonstrable before the treatment with Lloyd's.

It is interesting to read what Behre and Benedict(1922) have to say about the presence of interfering substances in the determination of creatin. The estimations were performed on heat-coagulated filtrates. After hydrolysis of the filtrate with hydrochloric acid and lead, treatment with kaolin was carried out. The chromogenic material(presumably creatinin) was eluted from kaolin and determined colorimetrically. Referring to the blood of nephritics, they said, " our results have been so contradictory that we are led to believe that some of these bloods contain large quantities of one or more interfering substances in the creatine determination, while in others the creatine determination may be fairly exact."

Gaebler and Keltch(1928) likewise reported difficulty in obtaining harmonious results with the method of Behre and Benedict. They determined blood creatin in a nephrectomized dog and a nephritic patient. Then they isolated creatin from these filtrates by their method. By isolation they obtained

higher values than by the other method.

Greenwald and McGuire (1918) expressed dissatisfaction with the apparently high values of creatinin and creatin obtained with picric acid filtrates (Folin method). Hence they devised their own method. The proteins were removed by precipitation with trichloroacetic acid in most of their analyses. The filtrate was then treated with kaolin to remove all of the creatinin. Next the filtrate was hydrolysed with hydrochloric acid to convert creatin to creatinin. A portion of the neutralized filtrate was then estimated colorimetrically for creatinin. This gave the value for creatin. Total creatinin presumably was determined by colorimetric analysis after hydrolysis of a portion of the filtrate without the preliminary use of kaolin. The authors do not state how the total creatinin was actually determined. The preformed creatinin was estimated by subtracting the creatin from the total.

Preformed creatinin was much lower by this method, which is what one might expect since in the Folin method the picric acid used to precipitate the proteins may react with the blood to form chromogenic substances. The creatin values were also lowered but not as much. In retention creatinin and creatin were increased , the latter being proportionally less (according to both methods).

The existence of creatin in blood is more problematical than that of creatinin, since its presence can only be satis-

factorily demonstrated after conversion to creatinin. Therefore, one can not prove that the creatinin thus formed came from creatin and not some other substance.

Normal and pathological variations of blood creatinin and creatin

A discussion of the normal variations in the preformed and total creatin of the blood must of necessity precede any discussion of the experimental changes occurring after the administration of cyanamid. The following values have been adopted from tables in Hunter (1928), pages 107 and 94. The normal whole blood creatinin in men and women varies from 0.96 to 2.80 mgm. per cent, averaging in the whereabouts of 1.0 to 1.5 mgm. per cent. Creatin is somewhat higher, ranging from 2.2 to 5.9 mgm. per cent and averaging about 4 per cent. Any higher values that have been reported for blood creatin are attributable to the use of picric acid in the hydrolysis of blood filtrates. Most of the higher values date back to 1922 or before, so that one can avoid the difficulty by arbitrarily excluding the figures collected before that time.

The acceptable values for rabbits taken from page 101 in Hunter (1928) are 1.0 to 1.7 mgm. per cent for preformed creatinin. The lowest values for creatin range from 3.7 to 4.6 mgm. per cent but are probably high.

Variations in blood creatinin

Although one ordinarily considers blood creatinin as constant, actually, it is subject to many variations. Blood creatinin is raised following the ingestion of creatinin (Cameron 1933), or its injection (Folin and Denis 1912); in human nephritis (Behre and Benedict 1922; Selman and Linegar 1933; Greenwald and McGuire 1918); in experimental nephritis or ligation of ureters (Behre and Benedict 1922) and in nephrectomy (Chanutin and Silvette 1929). It is not significantly changed in exercise (Rakestraw 1921) during menstruation (Wang and Dentler 1921) or pregnancy (Hellmuth 1923). At times, blood creatinin may fall to lower levels than normal. This has been reported as occurring to a slight extent in animals receiving injections of insulin or adrenalin (Rigo and Frey 1934). Moreover, according to Cardinale and Arnone (1935), the blood of individuals afflicted with muscular hypertonia contains extremely small concentrations of creatinin. On the other hand, no changes in blood creatinin have been found in muscular dystrophies (de N. Hough 1931), myasthenia gravis (Boothby 1932,), or myotonia atrophica (Morgulis 1931).

Variations in blood creatin

The creatin in blood is probably subject to more fluctuation than creatinin. It is easily raised in response to creatin injection (Folin and Denis 1912); proportionally less elevated than creatinin in nephritis, (Gavrila 1932);

and raised in renal suppression (Behre and Benedict 1922; Chanutin and Silvette 1929). It is strikingly raised following injections of guanidin carbonate in dogs (Zappacosta 1935). A slight rise is demonstrable after exercise (Kácl 1932) but not according to Rakestraw (1921). It has been shown to fall slowly in fasting dogs and rise as the fast is prolonged (Morgulis and Edwards 1924); and also rise in fasting rats (Chanutin and Silvette 1928). Subsequent refeeding allows a return to the normal value in dogs (Morgulis and Edwards 1924), but is responsible for a sharp rise the first day of realimentation in rats (Chanutin and Silvette 1928). Blood creatin undergoes a fall after injections of insulin or adrenalin (Rigo and Frey 1934). Looney 1924,1) observed a fall in blood creatin in cases of dementia praecox with muscular relaxation, and a rise in catatonic rigidity (Looney 1924,2). In one case of myasthenia gravis, low values for blood creatin have been reported (Boothby 1932,).

From the above resume, one can conclude that blood creatinin is only altered significantly in renal disease. Except for the work of Cardinale and Arnone, the creatinin in blood does not vary in muscular disturbances.

On the other hand, blood creatin undergoes considerable changes in starvation, is raised after the injection of certain drugs (guanidin), and lowered after the injection of others (insulin or adrenalin). Finally, blood creatin exhibits striking variations in muscular diseases.

Variations in blood creatinin and urea after the injection of cyanamid

Raida (1923) injected 0.2 gml of cyanamid per kilo subcutaneously into a rabbit, withdrew 0.5 cc. of blood from the ear at hourly intervals, and determined urea and creatinin by Folin's methods on each specimen (small as it was).

The blood urea nitrogen rose from 21 to 59 mgm. per cent in three hours and fell to 42 mgm. per cent in five hours (expressed by Raida, Table 2, as urea in mg.%).

Creatinin rose from 1.0 to 3.0 mgm. per cent in four hours and dropped to 2.4 mgm. per cent at the end of five hours (expressed by Raida as 10, 30 and 24 mg.%, respectively). Raida does not include any control figures. He merely states that the daily variations in blood urea and creatinin were found to be quite small. It should be noted that the blood urea and creatinin did not return to their normal levels at the conclusion of the experiment. No information is given concerning the recovery of the animal, although it is not likely that he did.

Table 2

Table from Raida Showing Changes in Blood Creatinin and Urea After the Injection of Cyanamid in a Rabbit.

Rabbit 2.1 kg.

Time 0.5 cc. blood taken from ear vein		Blood urea in mg.%	Blood creatinin in mg.%
7:00		21	10
8:00		21	11
9:00		27	19
10:00	0.2 gm. of cyan-	59	22
11:00	amid per kilo	59	30
12:00	subcutaneously	42	24

Variations in urinary creatinin and creatin after injections of cyanamid and dicyandiamid

Hesse (1921) injected several rabbits with cyanamid or dicyandiamid and studied the nitrogen partition in the urine.

In the first experiment he injected a rabbit subcutaneously with 0.15 gm. of cyanamid per kilo and collected urine daily for three days following the injection. Control urine was collected for three days preceding the experiment. Total nitrogen, urea, ammonia, creatinin and creatin were determined. The output of total nitrogen was 343 mgm. the day before the injection and rose to 561 the day following it. This difference in nitrogen excretion is adequately accounted for by the nitrogen of cyanamid. The average creatinin output for the three days preceding the experiment was found to be 52.3 as compared with the excretion of 38.0 mgm. for the three days following the injection. The creatin increased from 10.3 the day before the injection to 20.2 mgm. the following day. The rise in creatin immediately following the injection was more than offset by a fall in the excretion of creatinin or even total creatinin. Moreover, the animal suffered a 10 per cent loss in weight, which is frequently accompanied by an increased creatinuria. See Table 3.

Table 3

Table from Hesse Showing Changes in Nitrogenous Constituents of Urine After the Injection of Cyanamid in a Rabbit

Exp. 9; Rabbit 1900 gm.

(The values are given in mgm. unless otherwise noted.)

Date	Weight in gm.	Urine vol. in cc.	Total Nitrogen	Urea Nitrogen	Ammonia Nitrogen	Preformed Creatinin	Creatin
13.VI.	2000	33	427	356	0.9	66.3	5.6
14.VI.	1960	17	298	282	0.3	41.5	16.3
15.VI.	1960	25	343	312	0.8	49.0	10.3
Rabbit received 0.3 gm. cyanamid (200 mgm.) subcutaneously.							
16.VI.	1960	36	561	531	1.0	46.1	20.2
17.VI.	1840	24	290	258	0.6	33.6	7.2
18.VI.	1840	28	301	252	0.5	34.2	10.2

In the next experiment urine was first collected 5½ and 18½ hours before the injection, and at the same intervals following the subcutaneous administration of 0.16 gm. of cyanamid per kilo. Table 4 reveals no change in creatin and creatinin excretion5½ hours following the injection but a rise in both in the 18½ hour period. The average creatinin excretion for the 24 hours preceding the injection was 32.4 mgm. and increased to 43.5 mgm. in the 24 hour period following the injection. Moreover, there was also a rise in the output of creatin from 6.0 to 15.1 mgm. for the same period. The average excretion of creatinin or creatin for the two days after the administration of cyanamid was also slightly higher than their output in the same period preceding the injection.

In a third experiment 0.17 gm. of dicyandiamid per kilo was injected. The preformed creatinin excretion rose from an average value of 33.9 for the two days preceding the experiment to 48.1 mgm. for the 24 hours following the injection. The creatin excretion fell from 8.7 to 5.9 mgm. Although the changes in total creatinin output were negligible, the increase in preformed creatinin is significant, since the animal gained slightly in weight. See Table 5.

Summarizing the results of Raida and Hesse on animals, we can say that they suggest the existence in the body of the same relation of cyanamid to creatin and creatinin as was demonstrated by Volhard and others in vitro (page 39).

Table 4

Table from Hesse Showing Changes in Excretion of Total Nitrogen, Creatinin and Creatin After the Injection of Cyanamid in a Rabbit

Exp. 10; Rabbit 1880 gm.

(The values for nitrogenous components are in mgm.)

Date	Weight in gm.	Urine vol. in cc.		Total Nitrogen	Preformed Creatinin	Creatin
19.VI.	1840	after $5\frac{1}{2}$ hrs.	5.5	66	7.7	1.8
		" $18\frac{1}{2}$ "	18.5	224	25.9	5.4
			24.0	290	33.6	7.2
20.VI.	1880	after $5\frac{1}{2}$ hrs.	7.6	49	7.2	1.1
		" $18\frac{1}{2}$ "	23.4	162	24.0	3.8
			31.0	211	31.2	4.9
Rabbit received 0.3 gm. cyanamid (200 mgm. nitrogen) subcutaneously.						
21.VI.	1860	after $5\frac{1}{2}$ hrs.	11.0	69	8.7	1.3
		" $18\frac{1}{2}$ "	27.0	446	34.8	13.8
			38.0	515	43.5	15.1
22.VI.	1800	after $5\frac{1}{2}$ hrs.	5.5	52	8.2	1.8
		" $18\frac{1}{2}$ "	15.4	182	26.0	6.3
			20.9	234	34.2	8.1

Table 5

Table from Hesse Showing Changes in Excretion of Total Nitrogen Creatinin and Creatin After the Injection of Dicyandiamid in a Rabbit

Exp. 11; Rabbit 1860 gm.

(The values for the nitrogenous components are in mgm.)

Date	Weight in gm.	Urine vol. in cc.	Total Nitrogen	Preformed Creatinin	Creatin
16.VI.	1840	24	290	33.6	7.2
17.VI.	1840	28	301	34.2	10.2
	Rabbit received 0.3 gm. of dicyandiamid in sodium carbonate subcutaneously.				
18.VI.	1860	31.5	715	48.1	5.9
18.VI.	1881	30.0	211	31.2	4.9

Inasmuch as the writer studied tissue creatin, and particularly muscle creatin, in cyanamid poisoning, he considers it pertinent to discuss the factors involved in the determination of muscle creatin and the variations in tissue creatin.

Determinations of muscle creatin

Analyses of muscle creatin are usually performed on moist tissue. However, knowing the value of the moisture content, we can recalculate the values in several ways. Three ways will be described.

1. The creatin values can be corrected to a constant total solids content for both the control and experimental tissues (Heidermanns 1927).

2. The creatin values of the experimental tissue are corrected to the total solid content of the control tissue.

3. The creatin values are calculated on the basis of dry tissue, after their determination in the usual way on moist tissue.

In addition, the values obtained on fresh, moist tissue may be corrected for the presence of fat, ash and moisture, and thus expressed in terms of fat-free, ash-free, dry tissue(Chanutin 1930). Obviously the correct method of analysis is one which embodies the fewest number of corrections for the unknown factors which may influence the moisture content. The investigators who correct moist

tissue creatin values to a constant water content make the doubtful assumption that the concentration of creatin is independent of the concentration of water. In the following discussion muscle creatin is always understood to be in terms of moist tissue, unless otherwise mentioned.

Variations in muscle creatin

Muscle creatin varies under a host of circumstances. It undergoes changes in fasting; varies with the administration of different drugs; is modified by changes in pH; and is altered by the administration of creatin, creatinin, various amino acids and so-called precursors.

Effect of fasting on muscle creatin

Short periods of fasting increase the creatin content of rabbit muscle; long fasts lower muscle creatin according to Myers and Fine (1912). No attempt will be made to consider fully the changes occurring in prolonged inanition, because they are irrelevant to the discussion, and as yet incompletely understood. Mendel and Rose (1912) found that muscle creatin rose steadily throughout starvation in rabbits. Rose (1935) pointed out that the sum of the total body creatin and excretion at the end of a prolonged fast is much greater than the amount one would expect to find at the close of starvation, if creatin were continually lost from destroyed muscle. It is thus inferred in contrast with Myers and Fine, who believe muscle creatin is irreparably lost from the body in starvation, that there is a synthesis of creatin and

creatinin (in agreement with Mendel and Rose). Further proof that muscle creatin increases in starvation was furnished by the experiments of Chanutin (1930,2) on dry, fat-free tissue. "Analysis of muscle of animals that had lost from 30 to 45 per cent of body weight shows an increase of six per cent in the creatine content" (presumably dry, fat-free tissue). The creatin content of dry, fat-free muscle is thus much smaller than that of the moist tissue.

In short fasts there seems to be a rough correlation between the increase in muscle creatin and the concomitant loss in weight. In Myers' and Fine's experiments there was an average gain in muscle creatin of 8.0 per cent with a weight loss averaging 29.3 per cent for two rabbits, which had been starved six and seven days respectively. Mendel and Rose (1912) found qualitatively similar changes. Two rabbits that had been starved for seven and a half, and nine days suffered an average weight loss of 31.7 per cent with an increase in muscle creatin of 12.2 per cent (Mendel and Rose 1912). Rats that had been deprived of food for one day lost from 0.to 10 per cent of their body weight, underwent a rise in muscle creatin of only 3.9 per cent (Chanutin and Silvette 1928). Weight losses of 10 to 20 per cent were accompanied by increases of 9.7 per cent in muscle creatin (Chanutin and Silvette 1928).

It would therefore seem that a loss of 10

to 20 per cent in body weight in rats is concomitant with a greater change of muscle creatin than in rabbits.

Effect of administration of drugs on muscle creatin

Riesser (1916), Riesser and Brentano (1930), and Brentano (1930) reported on the changes in muscle creatin following the administration of various drugs to rabbits. Epinephrin, in doses of 2.0 to 2.5 mgm., subcutaneously injected, resulted in an average rise of 10 per cent in muscle creatin in three of Riesser's experiments. The controls were taken from a different series of animals.

Brentano in one experiment obtained a rise of 22 per cent in muscle creatin, three days following the intraperitoneal injection of 1 mgm. of epinephrin per kilo. The control value in this as in the other experiments of Brentano (1930), or Riesser and Brentano (1930), was obtained by analysis of the posterior tibialis muscle of one leg several days before the injection. No control experiments were performed to determine the effect of the removal of the muscle alone on the muscle creatin. Inasmuch as the animals lost weight after the removal of the control tissue or at some time between the beginning of the experiment and its termination, the changes in muscle creatin must be critically considered. A 10 to 20 per cent loss of weight was always accompanied by a rise in muscle creatin of about eight per cent in rats (Chanutin and Silvette 1928). Although it is not strictly correct to apply observations on rats directly to rabbits, yet one is justified in

assuming that a loss of over 10 per cent of body weight in rabbits would be followed by an increase in muscle creatin. For the purpose of comparison, we will deduct five per cent from the original values for muscle creatin whenever there is a loss in body weight of at least 10 per cent. Inasmuch as the rabbit which had been injected with epinephrine lost 15 per cent weight, we would therefore tentatively reduce the increase in muscle creatin from 22.2 to 17.7 per cent (Brentano 1930).

Injections of about 0.15 gm. of caffeine were followed by increases of 10 per cent in muscle creatin in three experiments (Riesser 1916).

The injection of 1.5 gm. of camphor per kilo in one rabbit was followed by severe spasms (Brentano 1930). Muscle creatin rose 21.8 per cent. Correction for accompanying weight loss of 17 per cent would reduce this value to 16.8 per cent.

Injections of picrotoxin, which also evoked convulsions, resulted in an average rise of 2.8 per cent in three rabbits (Riesser 1916). Apparently a significant increase in muscle creatin does not always accompany convulsions.

Injections of tetra-hydro-b-naphthylamine resulted in a marked rise in temperature and an increase of 15.5 per cent in muscle creatin in four animals (Riesser 1916). Paradoxically, if curare were given and the body temperature of the animals allowed to fall considerably, a rise of seven per cent of muscle creatin was observed in four rabbits

(Riesser 1916). Apparently, disturbances in the maintenance of body temperature would seem to have some effect on muscle creatin.

The administration of thyroid or thyroxin in rats produced alterations in muscle creatin. Dessicated thyroid caused an average fall of nine per cent (Bodansky 1935).

Effect of acidosis on muscle creatin

Rabbits which had been injected with ammonium chloride developed marked acidosis (Riesser and Brentano 1930); and Brentano 1930). The writer has considered only those experiments in which the pH of the blood fell at least 0.05 units, and changes in body weight were recorded. Most of these experiments cover a period of a week or so. The fall in pH varied between 0.09 and 0.47; and the loss in weight averaged 11 per cent in six rabbits, in which there was a rise in muscle creatin of 10 per cent. Reduction for the loss in weight still does not rule out a rise of five per cent in muscle creatin. It is difficult to say that acidosis per se is responsible for the increase in creatin, since there is no great change in muscle creatin in nephritis which is accompanied by acidosis.

Effect of alkalosis on muscle creatin

A fall in muscle creatin occurred in some of the experiments in which alkalosis was produced by the injections of sodium bicarbonate (Riesser 1930; Brentano and Riesser 1930).

Again the writer has considered only those experiments in which the change in pH was more than 0.05 units and in which the data on weight were recorded. The muscle creatin in two experiments fell 11 per cent in two experiments. Since muscle creatin usually rises when body weight falls, the usual correction for weight loss should raise these values to 16 per cent. It would thus appear that in alkalosis due to the injection of bicarbonate, muscle creatin is diminished. Curiously enough, however, a rise in muscle creatin was observed in the experiment with epinephrin where an increase in the pH of the blood occurred (Brentano 1930).

Effect of the nervous system on muscle creatin

Denervation of one of the limbs seemed to diminish the rises in muscle creatin obtained in animals subjected to the influence of various drugs. For instance, in two experiments with epinephrin, the muscle creatin of the denervated leg exhibited a rise of 4.8 per cent, while that of the intact leg increased by 8.0 per cent. Similarly in two experiments where cooling induced an increase of 6.8 per cent, the creatin in the denervated limb rose only 2.9 per cent (Riesser 1916).

Riesser concluded from these experiments that the nervous system played an important part in creatin metabolism.

Effect of creatin-creatinin administration on muscle creatin

Myers and Fine (1913) injected several rabbits with 0.7 to 1.2 gm. of creatin daily for five to 13 days and found

that the muscle creatin increased about five per cent. The animals were killed one to four days after the last injection. The sum of the muscle creatin plus the total output of creatinin and creatin, which were also determined, left unaccounted from 0 to 52 per cent of the injected creatin.

Creatinin was administered in another set of experiments. Similar rises in muscle creatin were observed, but most of the remaining creatinin could be accounted for by the output in the urine (Myers and Fine 1913).

The destruction of creatin or its storage elsewhere in the body might possibly explain the lack of greater changes in muscle creatin after the administration of creatin or creatinin.

Chanutin (1927) studied the changes in the creatin content of muscle and other tissues in rats subsisting for two months on diets containing 0.67 to 2.67 per cent creatin. Muscle creatin rose only 3.8 per cent in the rats on the higher creatin diet.

Adult rats maintained for 19 days on a diet containing five per cent of creatin exhibited rises of 19 per cent in muscle creatin. However, if young rats were kept on diets containing 10 per cent of creatin for two days, the rise was 236 per cent. This would indicate that the young rat is the more susceptible to changes in muscle creatin (Chanutin and Silvette 1928).

If adult rats were kept on a diet containing 10 per cent of creatin they exhibited a rise of 14 per cent in the muscle creatin only on the first day of feeding. The values on the second day were four per cent higher than the normals. Apparently the saturation value for muscle creatin was reached the first day.

Administration of 100 mgm. of creatin by stomach tube to adult rats produced no significant rise in muscle creatin in three to six hours. Twenty-one per cent of the creatin given was recovered from the alimentary tract (Bodansky 1936).

On the other hand, Beard and Barnes (1931) obtained increases of 23.6 per cent in muscle creatin 17 to 24 hours after feeding young rats 0.5 to 1.0 gm. of creatin. Beard and Bogges (1936) reported experiments in which the adult rats were sacrificed one to four days following the intraperitoneal injection of 100 mgm. of creatin. The average increases in muscle creatin were 17.4 per cent. By way of summary we might say that the changes observed in muscle creatin of rats vary with the age of the animals and the mode and length of administration of creatin.

Effect of administration of substances related to creatin

Several substances will be considered in this section, viz, guanidin, guanidin-acetic acid, arginin, glycin, choline and betaine. Wishart (1919) performed creatin determinations on the gastrocnemius muscle following the subcutaneous or intravenous administration of guanidin salts. One of the

gastrocnemii was removed under anesthesia or after decerebration; the guanidin was injected and the animals were killed when the neuromuscular symptoms had become quite marked (usually one or two hours later. Similar increases in muscle creatin were observed in frogs, cats and dogs. In one cat the rise was 20.4 per cent after the administration of 0.2 gm. of guanidin carbonate. An increase in muscle creatin of 21 per cent occurred in one dog following the injection of 0.86 gm. of guanidin-sulphate given in several portions.

Wishart's experiments should be repeated with adequate controls. However, it is not likely that the operative procedures contributed to the changes in muscle creatin according to the control experiments of Thompson (1917) in his studies of arginin.

Average increases of 8.5 per cent in muscle creatin in five rats were observed two days following the injection of 50 to 100 mgm. of methylguanidin. No change was observed in the muscle creatin of the animals that died the day of the injection (Beard and Bogges 1936).

Baumann and Hines (1917) could not detect an increase in muscle creatin after the injection of guanidin-acetic acid (glycocyamine) in dogs, although they did observe an increased output of creatin. Perfusion of the hind leg with guanidin-acetic acid produced an increase of 10.4 per cent in muscle creatin (calculated on a dry basis). Due allowance was made for the chromogenic equivalent of the glycocyamine.

Bodansky (1936) administered 100 mgm. of guanidin-acetic acid by stomach tube to rats and observed no significant increase in muscle creatin. However, an increase in excretion of creatin occurred.

Beard and Barnes (1931) reported rises of 48.5 per cent in muscle creatin in young rats after feeding one gm. doses of glycocyamine. Beard and Bogges (1936) observed an average rise of 14.9 per cent in adult rats following several intraperitoneal injections of 100 mgm. of glycocyamine. The chromogenic effect of the guanidin-acetic acid was not considered in any of these experiments.

Arginin has long been considered as one of the possible precursors of creatin. Thompson's experiments (1917) were numerous and carefully performed, but not confirmative of the above hypothesis. He observed an increase of 2.4 per cent in muscle creatin of 9 rabbits after the intravenous injection of arginin. The injections were made directly following the removal of the control leg. The other leg was removed for analysis six hours later. Controls with saline in place of arginin were negative.

Young rats maintained for 53 days on diets low in arginin suffered no decrease in tissue creatin (Meyer and Rose 1933). However, the oral administration of large doses of arginin in rats produced rises in muscle creatin of 29 per cent (Beard and Barnes 1931). Injections of 100 mgm. of arginin in rats also produced similar increases in muscle creatin (Beard and Bogges 1936).

Beard and Barnes (1931) reported rises of 15.4 per cent in muscle creatin of young rats which had been fed 0.5 to 1.0 gm. of glycin for one day. Intraperitoneal injections of 100 to 200 mgm. of glycin in rats produced increases in muscle creatin ranging from 5 to 32 per cent (Beard and Bogges 1936). The studies of Beard and Barnes, and Beard and Bogges on glycocyamine, arginin and glycin, are subject to the same criticism as their experiments with substances unrelated to creatin. (See below)

According to Riesser (1913), injections of 2.5 gm. of choline in rabbits produced rises of six to seven per cent after 24 to 48 hours. Some of the animals died after the injections.

Doses of 4.8 to 12 gm. of betaine hydrochloride injected in rabbits during 12 to 48 hours were followed by increases in muscle creatin averaging 8.6 per cent (Riesser 1913).

Effect of administration of substances apparently unrelated to creatin

Beard and Barnes (1931), and Beard and Bogges (1936) have presented evidence that amino acids such as valine, alanine, leucine, etc., which bear no relation chemically to creatin, increase muscle creatin in the rat.

These substances were fed in doses of 0.5 to 1.0 gm. to young rats of 40 to 55 gm. Increases in muscle creatin were of the same magnitude or greater than those observed after the feeding of creatin (page 74). Interpretation of the results in these feeding experiments is difficult. Firstly, the animals were young; secondly, their rations were removed until

the substance administered was consumed; thirdly, the doses were unphysiologic to say the least. In regard to the experiments of Beard and Barnes, Rose (1935) says," without questioning the accuracy of the author's observations, one is justified in assuming that the results are not to be attributed to a direct transformation of the compounds into creatine or creatinine." The reason for suggesting stimulation as the cause of the enormous changes observed is that each of the compounds studied were dissimilar and yet had the same effect.

The effect of several parenteral injections of these amino acids and related substances, are likewise subject to the same criticism (Beard and Bogges 1936).

Repetition of these experiments with smaller dosages might conceivably yield different results.

Effect of renal damage on muscle creatin

Rats nephrectomized and then fasted for two days exhibited a fall of 4.6 per cent in muscle creatin. This could be due to increased destruction of creatin, a shift of creatin to other organs, or the accumulation of water in the muscle Chanutin and Silvette 1929). The third factor is probably the most important since the total creatin content of the organism is not lowered two days after nephrectomy(Chanutin 1930,1). A shift of creatin from muscle to liver is precluded by the small increase in the creatin content of the latter organ after nephrectomy.

In 1916, Denis made an extensive study of the creatin

content of human muscle in various diseases. The normal value obtained by analysis of the muscle of five individuals dying from hemorrhage was 389 mgm. per cent. The average muscle creatin of 13 patients dying from various types of nephritis (arteriosclerotic, chronic, tuberculotic, etc.) was 395 mgm. per cent. This difference is insignificant. We can conclude on the basis of these observations that muscle creatin does not vary to a large degree in nephritis.

Muscle creatin in muscular diseases

It is interesting to note that the concentration of muscle creatin is markedly diminished in advanced muscular atrophy. Bodansky et al (1930) made an intensive study of a case of myositis fibrosa. Although blood creatinin and creatin were apparently normal, the muscle creatin was considerably diminished. Goettsch and Brown (1932) observed low values for muscle creatin in experimental nutritional dystrophy of dietary origin.

Variations in liver and kidney creatin

No attempt will be made to discuss fully all the changes that may occur in liver and kidney creatin.

Normal liver of rats contains 33 mgm. per cent of creatin; kidney, 46 mgm. per cent (Chanutin 1927). Liver creatin decreased 12 per cent in rats fasted until they had lost 0 to 10 per cent of their weight. The creatin content of the kidney in these experiments was increased by 40 per cent.

Starvation with a loss of 10 to 20 per cent in weight resulted in a five per cent fall in liver creatin and a 40 per cent rise in kidney (Chanutin and Silvette 1928).

The estimable creatin of the liver and kidney of rats maintained for two months on diets containing 2.67 per cent creatin, was elevated 176 and 57 per cent, respectively.

Liver creatin rose 400 to 500 per cent in rats one to two hours after receiving 100 mgm. of creatin by stomach tube (Bodansky 1936). In the same experiments kidney creatin attained values 300 to 400 per cent higher than normal.

The creatin content of the liver in rats was increased 90 per cent two days after nephrectomy (Chanutin and Silvette 1929).

The liver of normal rabbits has between 24 to 27 mgm. per cent creatin. The creatin content of kidney is between 19 and 20 mgm. per cent (Rose, Helmer and Chanutin 1927). Not many observations on the changes in liver and kidney creatin of rabbits have been made.

The writer undertook to redetermine the subcutaneous, minimum lethal dose of cyanamid in mammals inasmuch as there was considerable disagreement in the results of other investigators (Table 1, page 15).

Moreover, the data of Raida (1923) and Hesse (1921) tended to show an intimate relation between cyanamid and creatin. For this reason, analyses of the creatin and creatinin content of blood and urine and total creatin of various tissues were performed in rabbits that had been injected intravenously with lethal doses of cyanamid.

Experiments

Purification of cyanamid

The cyanamid used in the following experiments was purified from a preparation secured from the Eastman Kodak Company. The original product melted from 38.5° to 44.5° C., had a geraniol-like odor, and yielded approximately 80 per cent cyanamid (see method on page 5).

The purification was accomplished by extracting the material with several portions of ether, evaporating the combined extracts to dryness in vacuo, and recrystallizing the residue from ether. The cyanamid thus prepared was odorless and melted between 39.5° and 44.3° C. The accepted melting point is 44°C.

Twenty mgm. of this material (one cc. of a two per cent aqueous solution) when analysed according to the usual

methods contained 99 per cent of cyanamid. This solution was used for the experiments on mice to be discussed shortly. The percentage of cyanamid was determined in the same manner from time to time during a period of nine to ten weeks. Its concentration only decreased to 96 per cent in this interval.

Experiments on mice

Each of six full-grown mice (No. 1 to 6) was injected with 0.4 mgm. of cyanamid per gm. Respiration immediately became deeper and accelerated. Most of the mice were paralysed (i.e., unable to right themselves) within an hour after the injection. Hyperexcitability of the start reflex was also noted. Two hours after the injection it was possible to induce convulsive movements or spasms by pinching. It was also observed that the respiration had become slow and labored. Mice that were prostrate at this time died within three and a half to six hours. All of the others died within 31 to 45 hours (see Table 6). The animals exhibited cyanosis as a result of the respiratory embarrassment.

At autopsy, gas and yellowish fluid were present in the small intestine. Tracheal hemorrhages were noted in most of the animals. Histological sections of mouse No. 3 were made. The ileum exhibited non-uniform autolysis and desquamation of the mucous membrane or entire villi. There was no evidence of inflammation. The liver presented acute and chronic periportal inflammation.

The sinusoids of the spleen were dilated and engorged,

suggesting cardiac failure. There was also evidence of red cell destruction.

The tracheal hemorrhage that was observed grossly proved to be an artefact on microscopic examination. This does not, of course, mean that the tracheal hemorrhages in the other mice were also artefacts. The other organs did not exhibit any acute changes that could be referable to cyanamid, and are therefore not described. According to these findings death was due to cardiac failure.

The next set of six animals (No. 7 to 13) was injected with 0.2 mgm. of cyanamid per gm. of body weight. They also exhibited the same respiratory changes shortly after injection as did the previous group. None of them became paralysed. They were all alive and appeared normal eight days later, at which time they were used in the following experiment. A dose of 0.3 mgm. per gm. was administered and the usual prompt respiratory effects were again noted. In general all the animals behaved in the same way as did the group receiving 0.4 mgm., although the development of the poisoning was somewhat slower because of the smaller dosage. Individual treatment of these animals will be omitted since the cardinal changes are tabulated in Table 6. Five of the six animals died within three days. Number 12 survived and was used for further experiments.

About eight weeks later another series of animals (No.13 to 15) was injected with 0.3 mgm. of cyanamid per gm. Here,

however, but one of the three animals (No. 15) died. In fact, No. 13 was reinjected with 0.2 mgm. of cyanamid the next day and survived. It is thought that the lower mortality of this series might be due to three reasons. Firstly, we have the factor of individual differences; secondly, that of deterioration of cyanamid; and thirdly, in the previous series of 0.3 mgm. dosages, the animals had been injected with 0.2 mgm. of cyanamid per gm. a few days before.

Analysis of the cyanamid solution a few days later revealed a concentration of 96 per cent of cyanamid, hence its deterioration could not have been the reason for the difference observed. The other two factors can not be disposed of.

These experiments, in general, substantiate the minimum lethal dose of 0.3 and 0.4 mgm. per gm., and the respiratory changes, hyperexcitability, paralysis, and spasms (Coester 1896). The gas and yellow fluid in the intestine and the frequent occurrence of diarrhea confirm the results of Koelsch (1914;1916,2) and the experiments of Stritt (1909) and Hesse (1921) with rabbits. Hemorrhages in the trachea were observed in many of the mice, in agreement with Koelsch's observations on rabbits. The absence of paralysis in the series receiving 0.2 mgm. of cyanamid is in disagreement with Coester's observation that one-third of the m.l.d. always caused transient paralysis.

Injection of cyanamid and glycin in mice

Six mice (No. 16 to 21) weighing between 20 and 31 gm.

were subcutaneously injected with about two mgm. of glycin. They exhibited extensor spasms and rolling movements in a few minutes. Forty minutes later mice No. 16, 19, and 20 received 0.3 mgm. of cyanamid per gm. subcutaneously. By this time No. 17 had already died from the glycin alone. No. 16, 19, and 20* died within one and a half hours, whereas the two remaining glycin controls survived one hour longer (see Table 7). Apparently, the dose of glycin was too large for the animals to tolerate. In the next experiment smaller amounts of glycin were injected.

In the following series of mice (No. 21 to 24) approximately 0.2 mgm. of glycin per gm. was administered to each mouse, and immediately thereafter 0.3 mgm. of cyanamid per gm. was injected into No. 21 and 24. The two controls suffered no ill effects from the glycin alone, whereas the other two mice, which had also received cyanamid, succumbed within 17½ hours (seeTable 8).

We can conclude from these experiments, few as they are, that glycin does not protect mice against the toxic effects of cyanamid, since the survival period was not increased. This is in agreement with Hesse (1921).

*Dr. Tum Suden of the Department of Physiology verified the presence of cataracts which the writer had noted directly after death in mouse No. 20.

Table 6

Cardinal Signs of Cyanamid Poisoning in Mice

Mouse No.	Dose of Cyanamid	Respiratory Acceleration	Respiratory Depression	Paralysis	Spasms if pinched	Diarrhea	Gas, Yellow fluid in Intestine	Time of death in hours
1	0.4 mgm.	+	+	+	+	+	+	31
2	0.4	+	+	+	+	o	+	31-45
3	0.4	+	+	+	+	o	+	$3\frac{1}{2}$
4	0.4	+	+	+	+	+	+	6
5	0.4	+	+	+	+	+	+	31-45
6	0.4	+	+	+	+	o	+	$4\frac{1}{2}$
7	0.2	+	-	-	+	o		Recovered
8	0.2	+	-	-	-	o		"
9	0.2	+	-	-	-	o		"
10	0.2	+	-	-	-	o		"
11	0.2	+	-	-	-	o		"
12	0.2	+	-	-	-	o		"
7	0.3	+	+	+	-	o	o	28
8	0.3	+	+	+	+	+	o	81-90
9	0.3	+	+	+	+	+	+	$31\frac{1}{2}$-48
10	0.3	+	+	+	o	o	o	$25\frac{1}{2}$
11	0.3	+	+	+	+	o	o	$5\frac{1}{2}$
12	0.3	+	-	-	-	o		Recovered
13	0.3	+	-	o	o	o		"
14	0.3	+	-	o	o	o		"
15	0.3	+	+	+	-	o	o	$16\frac{1}{2}$

+ Positive
- Negative
o Not observed

Table 7

Time of Death After Administration in Mice of Glycin (large dose) and Cyanamid

Mouse No.	Dose of Glycin	Dose of Cyanamid	Time of Death in Hours
16	2 mgm.	0.3 mgm.	$1\frac{1}{2}$
17	2	---	Dead before injection with cyanamid
18	2	---	$2\frac{1}{2}$
19	2	0.3 mgm.	$1\frac{1}{2}$
20	2	0.3 "	Within $1\frac{1}{2}$
21	2	---	$1\frac{1}{2}$

Table 8

Time of Death After Administration in Mice of Glycin (small dose) and Cyanamid

Mouse No.	Dose of Glycin	Dose of Cyanamid	Time of Death in Hours
21	0.2 mgm.	0.3 mgm.	Within 17½
22	0.2 "	---	Survived
23	0.2 "	---	"
24	0.2 "	0.3 mgm.	Within 17½

Chromogenic reaction of cyanamid with alkaline picrate

Inasmuch as the writer intended to determine creatinin in body fluids and tissues of animals poisoned with cyanamid, it was thought advisable to ascertain whether cyanamid alone would develop a color with picric acid in alkaline solution. It so happened that cyanamid did have some chromogenic effect, but as will be shown this was not significant. The color developed by one mgm. of cyanamid, plus a definite quantity of picric acid and sodium hydroxide, exactly matched a solution of alkaline picrate without cyanamid. Moreover, heating cyanamid with picric and hydrochloric acids likewise did not produce any more color than the blank.

However, a slight amount of color developed in a solution containing from two to 25 mgm. of cyanamid treated with five to ten times the amount of alkali used in the determination of creatinin in blood. This quantity of cyanamid was thus estimated to be equivalent to 0.03 mgm. of creatinin. A mixture of one mgm. of glycin, one mgm. of cyanamid and the same proportions of picric acid and alkali used in the determination of creatinin, gave no chromogenic effect. Excess of alkali again caused the development of a slight color.

One mgm. of glycin and one mgm. of cyanamid were placed in a flask for one week. At the end of this time the usual proportions of picric acid and alkali were added but no color developed.

A solution of two mgm. of glycin, two mgm. of cyanamid

autoclaved in the presence of picric and hydrochloric acids, still produced no observable chromogenic change.

An ammoniacal aqueous mixture of one mgm. each of glycin and cyanamid, with or without a small amount of alcohol, gave no appreciable Jaffé reaction.

The last three experiments were performed because of the reaction of methylglycin (sarcosin) plus cyanamid to form creatin (pages 39-40).

It was therefore concluded that cyanamid, at room temperature or autoclaved, with or without glycin did not develop any significant chromogenic effect when treated with alkaline picrate as in the determination of creatin. Moreover, the possible colorimetric effect of cyanamid is only of theoretical importance, because Raida (1923) was at no time able to detect cyanamid in blood or tissues a few hours after the administration of cyanamid.

Experiments on rabbits

The following studies are divided into two parts, which will be discussed separately, although the experiments were carried out simultaneously.

1. Observations on the behavior of rabbits following the intravenous administration of lethal doses of cyanamid.

2. Determinations of preformed creatinin and total creatinin of the blood and urine: analyses of total creatin as creatinin of muscle, liver and kidney.

Methods and experimental technique

The chemical methods employed for blood creatinin and creatin were adapted from Folin (1934). The analyses were performed on tungstic acid filtrates. Purified picric acid instead of sodium picrate was used. It was found convenient to use the same creatinin standards for total creatinin as for the preformed creatinin, i.e., hydrochloric acid was omitted. This made no difference in the results as ascertained by comparisons with or without the acid. The standards used were 0.01 mgm. apart rather than 0.02 mgm., as called for in the original method. Mattice (1936) has recommended reading all the unknowns against a single standard, and then correcting for the deviation from Beer's law, by reference to a graph, the values for which had been obtained by reading known quantities of creatinin against one standard. However, it was found that satisfactory readings could be made by comparing the filtrates against standards, which matched fairly well by direct visual comparison.

Urinary creatinin and creatin were determined according to the methods of Folin. The procedure of autoclaving the urine in the presence of picric and hydrochloric acids invariably yielded high values for creatin. This was apparent from the rapidity of color development after the alkali had been added, and from the fact that the colors were not always of the same hue as pure creatinin standards or preformed creatinin of the urine. More direct evidence was

furnished by the observation that after autoclaving the urines with hydrochloric acid alone, the colors developed slowly and closely resembled the creatinin standards. Lower values were obtained by this method. The results are all comparable, even if higher than actual, since the procedure of using picric and hydrochloric acids was employed throughout the experiments.

Duplicate analyses were performed on creatin and creatinin of the blood and urine.

The method of Rose, Helmer, and Chanutin (1927) proved satisfactory for the estimation of total creatin in tissues.

In addition, determinations of blood inorganic phosphate according to Walker and Huntsinger (1930), amino acid nitrogen by Folin's method (Hawk and Bergeim 1931), and guanidin adapted from the method of Sullivan (1935) for guanidin in aqueous solutions or in amino acids were performed (Rabbits No. 12 and 13).

The cyanamid used in the experiments with rabbits was a 20 per cent aqueous solution of the purified material. A 20 per cent aqueous solution of glycin was also used in some experiments.

The experiments on rabbit No. 4, glycin injection; rabbit No. 5, control; and rabbits No. 7 and 8, cyanamid injections; were performed with the animal immobilized on an operating board the greater part of the experiment. A catheter was inserted into the bladder via the urethra at

the beginning of the experiment; the urine was discarded and the catheter was left in place for the duration of the experiment.

One hundred cc. of water was then given by stomach tube in the two experiments on rabbit No. 4 and the control experiment on No. 5. This procedure was omitted in the remaining experiments because of the possible relation of the ensuing diuresis and creatinin excretion.

At intervals of several hours the urine was removed in a graduated cylinder and set aside for analysis. This method was discontinued in the experiments on rabbits No. 9, 12, and 13, because the changes took somewhat longer to develop than was at first expected. In these experiments the animal was at first catheterized and the urine discarded. He was then placed in a cage to allow collection of normal urine for about 24 hours. At the end of the control period the animal was catheterized and the urine thus obtained combined with the cage washings. Urine was collected for a period of approximately 24 hours following the injection.

Control blood was drawn from the ear vein into an oxalated bottle. The bloods taken after the injection of cyanamid were, when possible, drawn from the ear, but in many cases were taken from the heart after stunning the animal by blows on the head. In one or two animals the final specimen was taken from the heart soon after the animal had died. Analyses on such bloods have been excluded from the

tables because of possible post-mortem changes.

Tissues for creatin analyses were taken as soon as the animal died from the effects of the cyanamid or immediately after stunning the animal. The specimens of muscle were always taken from the adductor longus muscle distal to the branching of the femoral vein. Samples of liver and kidney were taken at random.

In most of the experiments respiratory rate, heart rate, and rectal temperature were recorded before and after the injections.

At frequent intervals the writer observed the response of the rabbits to startling, or to slapping of the foot. Such stimulation of the animals that were kept tied down during the experiment often occasioned convulsive or spasmodic movements. However, some of these violent outbursts on the part of the animal were probably due to his efforts for freedom. They have been considered as spasms or convulsions whenever they were apparently spontaneous, or occurred in response to a definite stimulus such as slapping. The animals that were confined to cages during the experiment in order to collect 24-hour urines did not exhibit these convulsive movements to a great extent.

Thirteen experiments were performed on full-grown male rabbits. Rabbits No. 5 and 4 were used twice. In the first experiment, No. 5 was used as a control without receiving any injection, and at a later date injected with cyanamid.

In view of the relation between glycin and creatin (pages 39 to 40), rabbit No. 4 was injected with glycin in the first experiment and a few days later received injections of glycin and cyanamid. Rabbits No. 7, 8, 9, 12, and 13 were injected with cyanamid only. Inasmuch as the changes in blood and urinary creatinin resembled those occurring in renal insufficiency, the ureters of rabbits No. 15 and 18 were ligated and the changes in blood creatin-creatinin studied. Moreover, the animals that had been injected with cyanamid did not partake of food and water, so two starvation experiments were carried out (No. 15 and 18). The results of these experiments are found in the following tables.

Table 9a

Changes in Blood Creatin and Creatinin in Control Rabbit

Rabbit No. 5; 3.76 kilo

(All values are expressed in mgm. per cent.)

Time Blood Taken	Preformed Creatinin	Total Creatinin	Creatin as Creatinin
2:30 P.M.*	1.4	3.6	2.2
Rabbit received 100 cc. of water by stomach tube after blood sampling			
6:30 P.M.* (4 hrs. later)	1.3	3.8	2.5
7:45 P.M.* (1 hr. 15 min. later)	1.2	3.8	2.6

*Filtrates were made on unlaked blood.

Table 9b

Changes in Urinary Creatin and Creatinin in Control Rabbit

Rabbit No. 5

(The values are given in mgm. unless otherwise noted.)

Time Urine Collected	Volume in cc.	Preformed Creatinin	Preformed Creatinin in mgm. per hr.	Total Creatinin	Creatin as Creatinin
2:50 P.M.	4	9.8	---	12.4	2.6
Rabbit received 100 cc. of water by stomach tube					
5:15 P.M. (2 hr., 25 min.)	30	23.7	9.5	26.9	3.2
7:35 P.M. (2 hr., 20 min.)	68	19.0	8.3	20.4	1.4

Table 10a

Changes in Blood Creatin and Creatinin After Intravenous Injection of Glycin in Rabbit

Rabbit No. 4; 3.36 kilo

(All values are expressed in mgm. per cent.)

Time Blood Taken	Preformed Creatinin	Total Creatinin	Creatin as Creatinin
10:45 A.M.	1.5	2.8	1.3
Rabbit received 100 cc. of water by stomach tube after blood sampling			
" " 0.3 gm. of glycin per kilo by ear vein at 11:25 A.M.			
12:45 A.M. (1 hr., 20 min. after injection)	1.4	2.8	1.4
2:50 P.M. (3 hr., 25 min. after injection)	1.1	2.9	1.8
6:15 P.M. (6 hr., 50 min. after injection)	1.4	2.6	1.2

Table 10b

Changes in Urinary Creatin and Creatinin After Intravenous Injection of Glycin in a Rabbit

Rabbit No. 4

(The values are given in mgm. unless otherwise noted.)

Time Urine Collected	Volume in cc.	Preformed Creatinin	Preformed Creatinin in mgm. per hr.	Total Creatinin	Creatin as Creatinin
11:10 A.M.	10*	---	---	---	---
Rabbit received 100 cc. of water by stomach tube " " 0.3 gm. of glycin per kilo at 11:25 A.M.					
12:50 A.M. (1 hr., 40 min.)	32	17.1	9.78	17.1	0.0
2:40 P.M. (1 hr., 50 min.)	+	---	---	---	---
6:00 P.M. (3 hr., 20 min.)	60 ++	18.6	5.7	18.9	0.3

* Not analysed
\+ Part of sample lost
++ Corrected for loss due to spilling about 1/6 urine

Table 11a

Changes in Blood Creatin and Creatinin After Intravenous Injection of Glycin and Cyanamid in Rabbit

Rabbit No. 4; 3.42 kilo

(All values are expressed in mgm. per cent.)

Time Blood Taken	Preformed Creatinin	Total Creatinin	Creatin as Creatinin
11:00 A.M.	1.3	3.2	1.9

Rabbit received 100 cc. of water by stomach tube after blood sampling
" " 0.3 gm. of glycin per kilo by ear vein at 11:30 A.M.
" " 0.25 gm. of cyanamid " " " " " " 11:40 A.M.

4:25 P.M. (4 hr. 45 min. after injection)	1.3	3.0	1.7
8:30 P.M. (8 hr. 50 min. after injection)	1.3	3.1	1.8
10:40 A.M. (24 hr. 10 min. after injection)	3.0	9.6	6.6
4:10 P.M. (29 hr. 40 min. after injection)*	4.8	---	---

Rabbit No. 4 died between 33 hr. 20 min. and 45 hr. 20 min. after the injection of cyanamid.

Weight of animal at death, 3.14 kilo (7.9 per cent loss)

*Insufficient blood for complete analysis

Table 11b

Changes in Urine Creatin-creatinin and Tissue Total Creatin After Injection of Glycin and Cyanamid in a Rabbit

Rabbit No. 4

(The values are given in mgm. unless otherwise noted.)

Time Urine Collected	Volume in cc.	Preformed Creatinin	Preformed Creatinin in mgm. per hr.	Total Creatinin	Creatin as Creatinin
11:20 A.M.	11.5*	---	---	---	---
Rabbit received 100 cc. of water by stomach tube " " 0.3 gm. of glycin per kilo at 11:30 A.M. " " 0.25 gm. of cyanamid/kilo at 11:40 A.M.					
4:30 P.M. (5 hr., 10 min.)	58.0	28.7	5.6	30.2	1.5
8:30 P.M. (4 hr.)	98.0	22.6	5.6	24.5	1.9
3:50 P.M. (19 hr., 20 min.)	45.0	23.9	1.2	30.2	6.3

Tissue Total Creatin as Creatinin (in mgm. per cent)	
Muscle	564.7
Liver	37.8

* Not analysed

Table 12a

Changes in Blood Creatin and Creatinin After Intravenous Injection of Cyanamid in a Rabbit

Rabbit No. 5; 3.76 kilo

(The values are given in mgm. per cent.)

Time Blood Taken	Preformed Creatinin	Total Creatinin	Creatin as Creatinin
10:15 A.M.	1.1	2.9	1.8
Rabbit received 0.3 gm. of cyanamid per kilo by ear vein at 2:20 P.M.			
3.00 P.M. (40 min. after injection)	1.2	3.2	2.0
8:30 P.M. (6 hr., 10 min. after injection)	1.9	3.6	1.7

Rabbit was killed 18 hr., 25 min. after the injection of cyanamid.

Table 12b

Changes in Urine Creatin-creatinin and Tissue Total Creatin After Injection of Cyanamid in a Rabbit

Rabbit No. 5

(Values given in mgm. unless otherwise noted.)

Time Urine Collected	Volume in cc.	Preformed Creatinin	Creatinin in mgm. per hr.	Total Creatinin	Creatin as Creatinin
Rabbit received 0.3 gm. of cyanamid per kilo*					
9:10 P.M. (6 hr. 50 min.)	115	57.5	8.4	62.7	5.2

Tissue Total Creatin as Creatinin +

Muscle	500.5
Liver	43.9

*These urine values are somewhat high because the bladder was not completely emptied at the start of the experiment.

+The values for tissue creatin are questionable since the tissues were removed for analysis about 1½ hr. after death of the animal.

Table 13a

Changes in Blood Creatin and Creatinin After Intravenous Injection of Cyanamid in a Rabbit

Rabbit No. 7; 2.52 kilo

(The values are given in mgm. per cent.)

Time Blood Taken	Preformed Creatinin	Total Creatinin	Creatin as Creatinin
11:40 A.M.	1.7	2.3	0.6

Rabbit received 0.2 gm. of cyanamid per kilo by ear vein at 1:45 P.M.

6:00 P.M. (4 hr. 15 min. after injection)	3.0	3.3	0.3

Rabbit died between 6 hr., 15 min. and 6 hr., 45 min. after the injection of cyanamid.

Table 13b

Changes in Urine Creatin-creatinin and Tissue Total Creatin After Injection of Cyanamid in a Rabbit

Rabbit No. 7

(All values are expressed in mgm. unless otherwise noted.)

Time Urine Collected	Volume in cc.	Preformed Creatinin	Preformed Creatinin in mgm. per hr.	Total Creatinin	Creatin as Creatinin
1:15 P.M. (3 hr., 50 min.)	14.5	17.1	4.5	18.0	0.9
Rabbit received 0.2 gm. of cyanamid per kilo					
5:30 P.M. (4 hr., 15 min.)	3.0	4.5	1.1	4.7	0.2
8:30 P.M. (3 hr.,)	2.0	1.7	0.6	1.8	0.1

Tissue	Total Creatin as Creatinin in mgm. per cent
Muscle	492.3
Liver	21.0

Table 14a

Changes in Blood Creatin and Creatinin After Intravenous Injection of Cyanamid in a Rabbit

Rabbit No. 9; 2.38 kilo

(The values are given in mgm. per cent.)

Time Blood Taken	Preformed Creatinin	Total Creatinin	Creatin as Creatinin
5:00 P.M.	0.8	3.2	2.4
Rabbit received 0.08 gm. of cyanamid per kilo by ear vein directly after blood sampling.			
5:25 P.M., next day (24 hr. 25 min. after injection)	1.0	3.1	2.1
Rabbit received 0.20 gm. of cyanamid per kilo directly after blood sampling.			
4:00 P.M., next day (47 hr. after injection)	4.0	8.4	4.4

Rabbit died 50 hr. after the first injection of cyanamid.

Table 14b

Changes in Urine Creatin-creatinin and Tissue Total Creatin After Injection of Cyanamid in a Rabbit

Rabbit No. 9

(Values given in mgm. unless otherwise noted.)

Time Urine Collected	Volume in cc.	Preformed Creatinin	Preformed Creatinin mgm. per	Total Creatinin	Creatin as Creatinin
4:00 P.M. (24 hr. 30 min.)	205	112.7	4.6	155.8	43.1
Rabbit received 0.08 gm. of cyanamid per kilo at 5:00 P.M.					
5:00 P.M. (25 hr. after first collection)	300*	93.2	3.7	135.0	32.8
Rabbit received 0.2 gm. of cyanamid per kilo at 5:25 P.M., next day					
3:15 P.M. (22 hr. 15 min. after second collection)	101	71.7	3.3	96.0	24.3

Tissue	Total Creatin as Creatinin in mgm. per cent
Muscle	537.2
Liver	57.0

All urine volumes large because of cage washings.

* Values corrected for loss of about 1/5 of the urine.

Table 15a

Changes In Blood Creatin and Creatinin After Intravenous Injection of Cyanamid in a Rabbit

Rabbit No. 12; 3.16 kilo

(The values are given in mgm. per cent.)

Time Blood Taken	Preformed Creatinin	Total Creatinin	Creatin as Creatinin
6:45 P.M.	1.4	5.6	4.2

Rabbit received 0.2 gm. of cyanamid per kilo by ear vein at 6:55 P.M.

12:00 A.M. (17 hr. 5 min. after injection)	2.9	7.5	4.6

Rabbit was killed 17 hr. 5 min. after the injection of cyanamid.

Table 15b

Changes in Urine Creatin-creatinin and Tissue Total Creatin After Injection of Cyanamid in a Rabbit

Rabbit No. 12

(Values given in mgm. unless otherwise noted.)

Time Urine Collected	Volume in cc.	Preformed Creatinin	Creatinin mgm. perhr	Total Creatinin	Creatin as Creatinin
4:45 P.M. (25 hr. 35 min.)	90	171.0	6.7	230.4	59.4

Rabbit received 0.2 gm of cyanamid per kilo by ear vein at 6:55 P.M.

11:30 A.M. (18 hr. 45 min. after first collection)	68	48.3	2.6	52.4	4.1

Tissue	Total Creatin as Creatinin in mgm. per cent
Muscle	565.2
Liver	41.2
Kidney	39.7

Table 16a

Changes in Blood Creatin and Creatinin After Intravenous Injection of Cyanamid in a Rabbit

Rabbit No. 13; 3.5 kilo

(The values are given in mgm. per cent.)

Time Blood Taken	Preformed Creatinin	Total Creatinin	Creatin as Creatinin
3:00 P.M.	1.2	3.9	2.7
Rabbit received 0.2 gm. of cyanamid per kilo by ear vein at 3:05 P.M.			
7:30 P.M, next day (27 hr. 25 min. after injection)	5.1	10.7	5.6

Rabbit was killed 27 hr. 35 min. after the injection of cyanamid.

Table 16b

Changes in Urine Creatin-creatinin and Tissue Total Creatin After Injection of Cyanamid in a Rabbit

Rabbit No. 13:

(Values given in mgm. unless otherwise noted.)

Time Urine Collected	Volume in cc.	Preformed Creatinin	Preformed Creatinin mgm. per hr.	Total Creatinin	Creatin as Creatinin
2:45 P.M. (23 hr. 30 min.)	450	151.5	6.5	184.5	33.0
Rabbit received 0.2 gm. of cyanamid per kilo by ear vein at 3:05 P.M.					
7:05 P.M. (27 hr. 20 min. after first collection)	100	35.5	1.3	47.0	11.5

Tissue	Total Creatin as Creatinin in mgm. per cent
Muscle	555.7
Liver	35.7
Kidney	41.0

All urine volumes large because of cage washings.

Table 17

Changes in Blood Creatin-creatinin and Tissue Total Creatin of a Rabbit After Ligation of Ureters

Rabbit No. 15; 2.97 kilo

(The values are given in mgm. per cent.)

Time Blood Taken	Preformed Creatinin	Total Creatinin	Creatin as Creatinin
11:10 A.M.	1.5	3.4	1.9
Both ureters ligated at 12:30 A.M. under ether			
4:30 P.M., next day (28 hr. after ligation)	16.2	18.9	2.7

Animal died at 4:40 P.M. (28 hr. 10 min.) after ligation of ureters.

Tissue Total Creatin as Creatinin

Muscle	506.5
Liver	60.3
Kidney	63.0

20 cc. of urine (catheter specimen before operation) contained 32.2 mgm. of preformed creatinin.

Table 18

Changes in Blood Creatin-creatinin and Tissue Total Creatin of a Rabbit After Ligation of Ureters

Rabbit No. 18; 1.84 kilo

(The values are given in mgm. per cent.)

Time Blood Taken	Preformed Creatinin	Total Creatinin	Creatin as Creatinin
12:30 P.M.	1.8	4.4	2.6
Both ureters ligated at 1:30 P.M. under ether			
3:30 P.M., next day (27 hr. after ligation)	7.0	11.6	4.6

Animal killed at 3:35 P.M. (27 hr., 5 min.) after ligation of ureters.

Tissue Total Creatin as Creatinin

Muscle	551.6
Liver	21.7

Weight of animal at death, 1.77 kilo (3.8 per cent loss)

Table 19

Changes in Blood Creatin-creatinin and Tissue Total Creatin of a Rabbit After Starvation

Rabbit No. 16; 2.97 kilo

(The values are given in mgm. per cent.)

Time Blood Taken	Preformed Creatinin	Total Creatinin	Creatin as Creatinin
11:15 A.M.	1.6	3.9	2.3
Animal deprived of food and water.			
1:30 P.M., next day (26 hr. 15 min.)	1.5	3.8	2.3

Animal killed at 1:45 P.M. (26 hr. 30 min.) after fast began.

Tissue Total Creatin as Creatinin

Muscle	538.5	(left adductor longus 534.0)
Liver	21.7	(right " " 543.1)
Kidney	17.4	

Weight of animal at death, 2.87 kilo (3.4 per cent weight loss).

Table 20

Changes in Blood Creatin-creatinin and Tissue Total Creatin of A Rabbit After Starvation

Rabbit No. 17, 3.135 kilo

(The values are given in mgm. per cent.)

Time Blood Taken	Preformed Creatinin	Total Creatinin	Creatin as Creatinin
12:45 P.M.	1.6	3.9	2.3
Animal deprived of food and water.			
12:45 P.M., next day (24 hr.)	1.8	3.9	2.1

Animal killed at 12:50 P.M. (24 hr. 5 min.) after fast began.

Tissue Total Creatin as Creatinin

Muscle	520.4
Liver	23.5
Kidney	19.5

Weight of animal just before death, 2.88 kilo (7.8 per cent weight loss)

Cyanamid poisoning in rabbits

1. Physiological changes

In general, the phenomena observed in rabbits were the same as those noted in mice. The following discussion applies to all the rabbits studied.

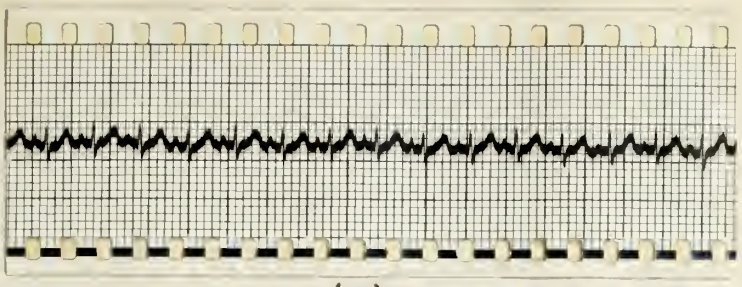
Immediately after the intravenous injection of 0.15 to 0.30 gm. of cyanamid per kilo the respiration became faster and deeper as well as abdominal. The electrocardiographs taken in rabbit No. 5 (Fig. 1) illustrate the heart changes that probably occurred in all the rabbits. The heart rate was considerably slowed 10 minutes after the injection. The rate continued to decrease for 25 minutes, but then slowly resumed its normal frequency within an hour after the injection. The auriculo-ventricular conduction time was not altered. The records offer no interpretation of the injury to the intraventricular automatic nervous system mentioned by Hesse (1921) (Page 12). The fine waves in some of the graphs were probably the result of tremors. Moreover, the variations in the base line may be attributed to movement of the animal.

The initial acceleration of the respiratory rate was soon followed by depression. The frequency of the respirations decreased; breathing became more difficult and slowed noticeably as the effects of the cyanamid increased. Respirations became infrequent, shallow and labored shortly before death.

It was difficult to observe exactly when paralysis took place, especially if the animals were tied. However, there was no doubt of its presence at the end of six hours. The criteria for paralysis were the inability of the animal to hold his head erectly, and to right himself when placed on the floor. Convulsive movements were observed somewhat earlier than the paralytic changes, but these were probably influenced by the immobilization of the animal and his struggle to escape. Touching or slapping the animal, or jarring the table nearby usually evoked these spasmodic movements (evidence of hyper-excitability). Pupillary reflexes were sluggish. After paralysis had set in, rhythmic progressive movements of the hind legs were exhibited by some of the animals when placed on the floor. A large fall in body temperature was noted in all the animals in which the rectal temperature was recorded, except rabbit No. 9. Tremors, shivering, and chattering of the teeth occurred frequently. Peristalsis was probably increased because the animals defecated more often. Watering of the eyes was exhibited by all of the animals. Table 21 reveals the major effects of the injection of cyanamid in rabbits.

At autopsy, the most common findings were the changes in the gastrointestinal canal. As in the mice, yellowish fluid and gas were present in the small intestine. The abdominal organs, particularly the liver and kidney, were hyperemic, except in rabbit No. 4. However, the spleen was usually contracted. There was no excess secretion in the trachea but petechial hemorrhages were present. Rigor mortis was marked. No other changes were revealed on gross examination.

Microscopical sections of various tissues were made on rabbits No.9,12, and 13. Necrosis, autolysis and inflammation of the mucosa of the small intestine were present in rabbit No. 9. Inflammation and venous congestion of the large but not the small intestine occurred in rabbit No. 12. Submucosal inflammation of the small intestine but not of the large intestine were present in rabbit No. 13. The lungs did not contain excess fluid in rabbit No. 9. The trachea of rabbits No. 12 and 13 revealed venous congestion and submucous edema. The liver of rabbit No. 12, but not of No. 13 exhibited passive congestion. The spleen revealed signs of heart failure in rabbit No. 9. The kidney was normal in rabbits No. 9 and 12, but exhibited cloudy swelling of the tubules, pyknotic nuclei and lightly staining cytoplasm in rabbit No. 13.



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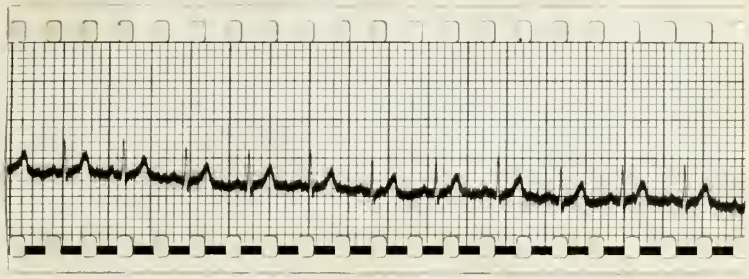

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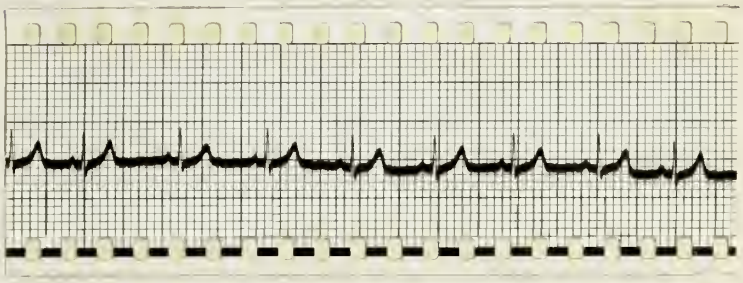

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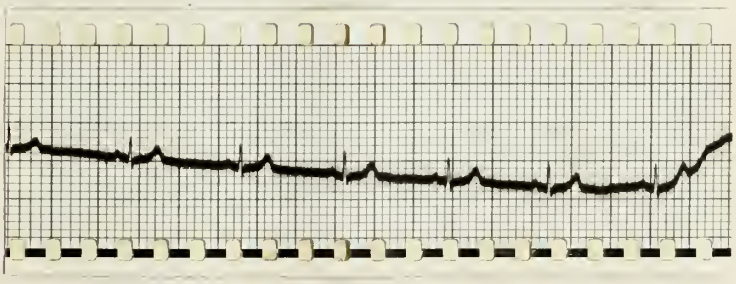

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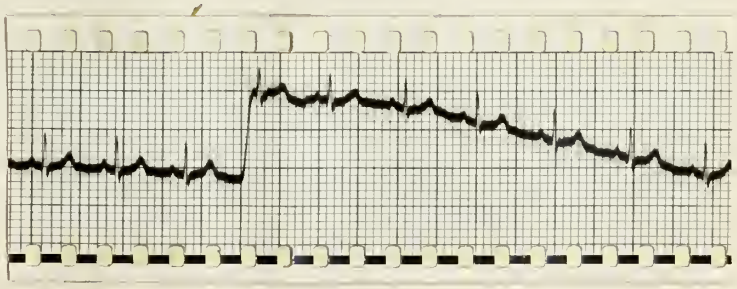
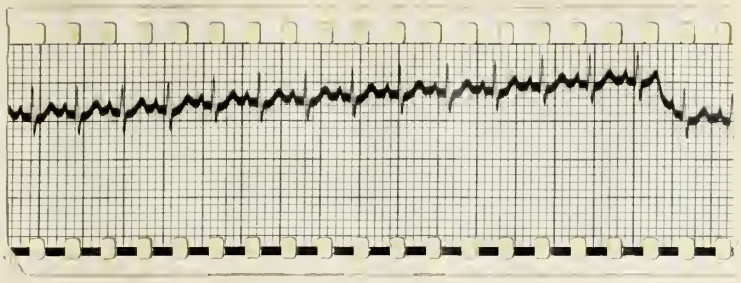


(e)

Fig. 9 (Continued)



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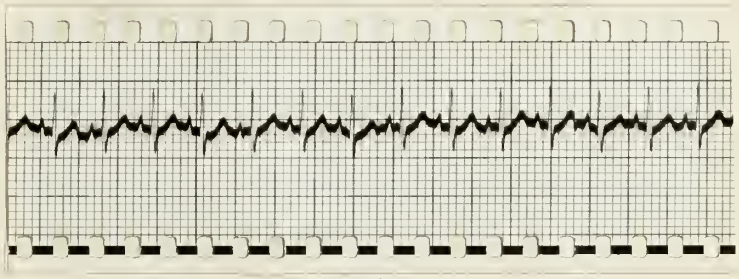

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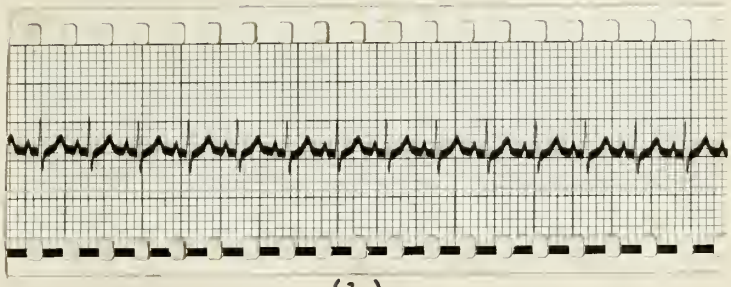

(h)

Fig. 9 Electrocardiographs of rabbit No. 5 before and at intervals following the injection of 0.3 gm. of cyanamid per kilo. All records taken with lead 1.

(a) before injection
(b) 10 minutes later
(c) 20 " "
(d) 25 " "
(e) 30 minutes later
(f) 60 " "
(g) 1 hour, 40 minutes later
(h) 2 " 15 " "

Table 21

Cardinal Signs of Cyanamid Poisoning in Rabbits

Rabbit No.	Dose of Cyanamid	Respiratory Acceleration	Respiratory Depression	Paralysis	Involuntary Spasms	Fall in Body Temperature	Gas, Yellow Fluid in Intestine	Time of Death in Hours
5	0.30gm.*	+	+	+	+	o	+	$18\frac{1}{2}$
4	0.25 "	+	+	+	+	4.3°C++	o	$33\frac{1}{3}$ - $45\frac{1}{3}$
7	0.20	+	+	+	-	5.4°C	+	$6\frac{1}{4}$ - $6\frac{3}{4}$
8	0.15	+	+	+	+	6.4°C	+	3
9	0.28	+	+	+	+	0.8°C (Rise)	+	50
12	0.20	+	+	+	+	o	+	17
13	0.20	+	+	+	+	5.4°C	+	$28\frac{1}{2}$

+ Positive
- Negative
o Not observed

*Animal also received 0.3 gm. of glycin in ear vein (Rabbit No. 5 only).
++The fall in temperature was recorded 22 hr. 20 min. before Rabbit No. 4 was found dead.

Animals No. 5, 12, and 13 killed by blows on head.

Discussion of physiological changes

The minimum lethal dose for cyanamid intravenously injected was found to vary between 0.15 and 0.30 gm. per kilo (rabbits No. 4, 5, 7, 8, 9, 12, and 13). This range of doses is smaller than Stritt's dose of 0.39 gm. per kilo for one rabbit and also smaller than Hesse's dose of 0.4 gm. (pages 10 and 13, respectively). These experiments support and amplify the results of Coester (1896), Stritt (1909), Hesse (1921), and Koelsch (1914; 1916,2). The writer observed slowing of respiration, muscular weakness, paralysis, spasms, clonic movements of the extremities, and a fall in body temperature, in agreement with the other investigators. In the following respects Coester's work was not confirmed. Dilatation of the ear vessels was not noted; constriction of the pupils occurred infrequently. Salivation was only noted in rabbit No. 9.

The conjunctivitis (watering of the eyes) observed by the writer also occurred in certain of the rabbits studied by Koelsch (1916,2). The variations in heart rate confirm and extend the findings of Stritt and Hesse. The occurrence of gas and yellow fluid in the intestine agree with the results of Stritt. The tracheitis observed confirms the work of Stritt, Hesse, and Koelsch. The writer believes that the tracheitis is due to the respiratory difficulty, but offers no explanation for the pathological changes in the gastro-intestinal tract.

2. Chemical Changes

Control experiment

Rabbit No. 5 was tied down on an animal board and treated in the same manner as rabbits No. 4, 5, and 7, except that no injection was made. The level of creatinin and creatin in the blood remained constant (Table 9a). The rate of creatinin excretion decreased about 12 per cent and the creatin, 53 per cent (measured in percentage fall of mgm. per hour excretion: Table 9b).

Glycin experiment

Rabbit No. 4 was intravenously injected with 0.3 gm. of glycin per kilo to determine the effect, if any, on blood creatinin and creatin. No appreciable changes occurred (Table 10a). The creatinin excretion fell considerably (42 per cent) during the experiment (Table 10b). The initial creatin excretion was negligible so that any slight changes that occurred were insignificant.

Starvation experiments

Rabbits No. 16 and 17 were starved 26 hr. 15 min. and 24 hr., respectively. The changes in blood creatinin and creatin were well within the experimental error(Tables 19 and 20).

Experiments with ligation of ureters

The blood creatinin rose 14.7 and 5.2 mgm. per cent, respectively, in rabbits No. 15 and 16, 28 hr. 10 min. and

27 hr., 5 min. after ligation of the ureters (Tables 17 and 18). The average rise in blood creatinin was 9.95 mgm. per 100 cc. (rise of 603 per cent). The blood creatin rose 0.8 and 2.0 mgm. per cent. The average rise in blood creatin was 1.4 mgm. per 100 cc. (rise of 62 per cent).

Cyanamid experiments

Rabbits receiving lethal doses of cyanamid showed increases in blood creatin-creatinin, falls in the excretion of creatinin (and creatin usually), rises in liver and kidney creatin, and possible rises in muscle creatin.

Variations in the creatin-creatinin content of blood

The changes in blood creatin-creatinin usually developed over a period of at least 17 hours, according to the rate of development of the changes in rabbit No. 4*. The following discussion does not include rabbits No. 5 and 7, since the last blood in these experiments was drawn 6 hr., 10 min., and 4 hr., 15 min., respectively, after the injection. Analyses on blood which was not taken immediately after death are also excluded since they are unreliable.

*About 10 days after the glycin experiment, rabbit No. 4 received each of 0.3 gm. of glycin and 0.25 gm. of cyanamid per kilo intravenously. The changes in blood were similar to those observed in the other cyanamid experiments. See Table 11a.

The rises in blood creatinin in rabbits No. 4, 9, 12, and 13, and rises in blood creatin in rabbits No. 4, 9, and 13 can be observed by referring to Table 22. The average rise in blood creatinin in the four experiments was 2.5 mgm. per 100 cc (rise of 206 per cent). The average rise in blood creatin in the four experiments was 2.6 mgm. per 100 cc. (rise of 94 per cent).

The author's average value of 1.5 mgm. per cent for blood creatinin in rabbits No. 4, 5, 9, 12, 13, 15, 16, 17, and 18 agree with those reported in the literature (page 56).

The average value for blood creatin is lower, however, than the reported ones. The blood creatin in the above-mentioned rabbits averages 2.5 mgm. per cent. The author explains this difference by the use of hydrochloric acid in the hydrolysis of the filtrate, rather than picric acid.

The blood guanidin was apparently increased while the phosphate fell to the point where it was not detectable (rabbits No. 12 and 13). However, the inhibiting effect of cyanamid on the color reaction between amino-naphthol sulphonic acid and phosphate may have been responsible for the great fall in blood phosphate after injection of cyanamid. The amino acid nitrogen rose in one case and fell in the other. These observations do not warrant further discussion because of their incompleteness.

Table 22

Changes in Blood Creatin-creatinin in Rabbits After Intravenous Injection of Cyanamid and After Ligation of the Ureters.

(All values are expressed in mgm. per cent)

Rabbit No.	Creatinin		Rise in Creatinin	Creatin	Rise in Creatin
4*	(Before injection)	1.3	1.7	1.9	4.7
	(24 hr., 10 min. after injection)	3.0		6.6	
9	(Before injection)	1.0	3.0	2.1	2.3
	(22 hr., 30 min. after injection)	4.0		4.4	
12	(Before injection)	1.4	1.5	4.2	0.4
	(17 hr. after injection)	2.9		4.6	
13	(Before injection)	1.2	3.9	2.7	2.9
	(27 hr., 30 min. after injection)	5.1		5.6	
	Average Rise		2.5		2.6
15	(Before Ligation)	1.5	14.7	1.9	0.8
	(28 hr., 10 min. after ligation)	16.2		2.7	
18	(Before ligation)	1.8	5.2	2.6	2.0
	(27 hr., 5 min. after ligation)	7.0		4.6	
	Average Rise		9.95		1.4

*Rabbit No. 4 also received 0.3 gm. of glycin per kilo.

Variations in creatin-creatinin of urine

The preformed creatinin excretion fell slowly in rabbits No. 4, 7, 9, 12, and 13. Table 23 reveals the changes in the rate of excretion of creatinin.* The hourly output fell considerably in rabbit No. 7 three hours after the injection, but not until about 19 hours after the injection in rabbits No. 4, 9, 12, and 13. The average fall in the excretion of creatinin in the five experiments was 3.8 mgm. per hour (fall of 68 per cent). In the control experiment No. 5, the fall was about 12 per cent.

The average creatin excretion in rabbits No. 4, 7, 9, 12, and 13 fell from 0.73 mgm. to 0.38 mgm. per hour. The average rate of creatin excretion fell 48 per cent in these experiments. The creatin excretion in the control experiment fell 35 per cent. Decreases in the average excretion of creatin were of little significance, since the initial creatin excretion was so small. In the experiments where blood creatin was considerably raised, the average rate of creatin output in mgm. per hour decreased 44 per cent (No. 4, 9, and 13). The creatin excretion was possibly slightly

*The total creatinin output for the 24-hour control period was in all cases considerably lower than the total excretion for the 24-hour period following the injection of cyanamid.

raised in rabbit No. 4.

Table 23

Changes in Rate of Creatin-creatinin Excretion After Intravenous Injection of Cyanamid in Rabbits.

(All values are average excretion in mgm. per hr. for the given period.)

Rabbit No.	Period	Creatinin	Fall in Creatinin	Creatin	Fall in Creatin*
4	5 hr., 10 min. after injection	5.6	4.4	0.29	---
	19 hr., 20 min. after injection	1.2		0.33	
7	3 hr., 15 min. before injection	4.5	3.9	0.02	---
	4 hr., 15 min. after injection	0.6		0.03	
9	24 hr., 30 min. before injection	4.6	1.3	1.7	0.6
	22 hr., 15 min. after second injection	3.3		1.1	
12	24 hr., 35 min. before injection	6.7	4.1	2.3	2.28
	18 hr., 45 min. after injection	2.6		0.02	
13	23 hr., 30 min. before injection	6.5	5.2	1.4	1.0
	28 hr., 20 min. after injection	1.3		0.4	
5 Control Rabbit	2 hr., 25 min. first collection	9.5	1.2	0.13	---
	2 hr., 20 min. second collection	8.3		0.06	

*The fall in creatin excretion is omitted in several cases because insignificant.

Variations in total tissue creatin

The average muscle creatin was 552.7 mgm. per cent in the rabbits injected with cyanamid (No. 9, 12, and 13). The average muscle creatin was 529.5 in the two starved rabbits (No. 16 and 17) and the two which had both ureters ligated (No. 15 and 18). The control values for tissue creatin were derived from the two rabbits which had their ureters tied and from the two starved rabbits. The increase in muscle creatin in the rabbits injected with cyanamid was 4.4 per cent. The liver creatin increased from 22.6 mgm. per cent in the two starved animals to 44.6 mgm. per cent in the rabbits injected with cyanamid. The rise in liver creatin was 97.3 per cent. The rise was but 8.6 per cent if the calculations were made with reference to the rabbits which had the ureters ligated.

The kidney creatin rose 119 per cent (using the starved animals as controls) but was lower than the kidney creatin of rabbit No. 15 (both ureters ligated).

Table 24

Changes in Tissue Total Creatin in Rabbits in Starvation After the Injection of Cyanamid and After Ligation of Ureters.

(All values are given in mgm. per cent.)

Rabbit No.	Muscle	Liver	Kidney
9	537.2	57.0	---
12	565.2	41.2	39.7
13	555.7	35.7	41.0
(Injections of cyanamid)			
Average:	552.7	44.6	40.3
15	506.5	60.3	63.0
18	551.6	21.7	---
(Ligation of ureters)			
Average:	529.0	41.0	63.0
16	538.5	21.7	17.4
17	520.4	23.5	19.5
(Starvation) Average:	529.9	22.6	18.4

Discussion of chemical changes

Changes in blood creatin-creatinin

In the introduction the writer stated that blood creatinin was increased in renal disease (page 57) and also after the injection of cyanamid in one experiment of Raida (page 60).

Inasmuch as there was evidence of kidney damage in cyanamid poisoning it becomes necessary to discuss this factor in detail. It has been definitely established in nephritis that the diminished excretion of creatinin is always accompanied by an elevation of blood creatinin.

In the author's experiments with cyanamid the increases in blood creatinin were accompanied by a decreased excretion of creatinin (rabbits No. 4, 7, and 12). The rate of excretion of creatinin in these three experiments fell 73 per cent. It was therefore logical to conclude that the injections of cyanamid resulted in damage to the kidney. As might be expected, the increase in blood creatinin after the injection of cyanamid was smaller than after ligation of the ureters, since the fall in urine excretion was not as great.

The mechanism whereby cyanamid disturbs kidney function is not clearly understood. Dittrich (1924), Glaubach (1926), and Kühnau (1927) have shown that cyanamid interferes with oxidation-reduction in the organism. It is not likely that the kidney would escape its influence. Another possibility is that the fall in blood pressure which developes as the effects of the cyanamid increases prevents adequate filtra-

tion pressure and thus results in decreased excretion of urine. It is the author's opinion that the rise in blood creatinin was due mainly to the interference with excretion of creatinin rather than to a transformation of cyanamid to creatinin or a chromogenic substance. The fact that the rise in blood creatinin in rabbit No. 9 was accompanied by a fall in excretion of only 30 per cent, suggests the possibility that the renal damage might not be the only cause for the increased concentration of blood creatinin.

The possible factors that might be responsible for the increases in blood creatin after the injection of cyanamid are renal injury, increased muscular tension (page 57), and increased formation of creatin from cyanamid. The relation of cyanamid to creatin was pointed out on page 39 of the introduction.

The changes in blood creatin in the rabbits injected with cyanamid were relatively and actually greater than those noted in rabbits after ligation of the ureters or in human nephritis.

The ratio of the percentage increase in blood creatinin to creatin was 2:1 about 24 hours after the injection of cyanamid, 6:1 in the cases of nephritis reported by Gavrila (1932), and 10:1 about 24 hours after ligation of the ureters.

The changes observed 48 hours after nephrectomy in rats (Chanutin and Silvette 1929) were similar to the author's results after the injection of cyanamid in rabbits.

These experiments are not comparable with those of the author because of species difference, and because of their long duration (48 hours). The greater instability of creatin metabolism in rats was pointed out in the introduction in a discussion of the changes in muscle creatin during starvation (page 68).

Blood creatin increased 94 per cent after the injection of cyanamid as compared with an increase of 62 per cent after ligation of the ureters, and 20 per cent in nephritis. The difference between the increase in blood creatin in cyanamid poisoning and after ligation of the ureters is considerably greater than the experimental error. Moreover, the degree of kidney damage in the latter experiments was undoubtedly greater than that produced by cyanamid. It is the author's opinion that greater differences might have been observed with controls that more closely reproduced the conditions in the rabbits injected with cyanamid.

The factor of increased muscle tension derives its importance from the considerable increases in blood creatin reported in several advanced cases of marked catatonic rigidity (Looney 1924;2) The author is inclined, however, to believe that the increased muscular activity exhibited in cyanamid poisoning was of insufficient duration and magnitude to be of considerable significance. The occurrence of paralysis in addition to spasms was observed in all the rabbits which had been injected with cyanamid. According to the

findings in dementia praecox with muscular relaxation (Looney 1924,1), this paralysis should have been accompanied by a fall in blood creatin. These experiments in general occupied a period of 24 hours. On the other hand, Looney's patients had apparently exhibited the state of increased muscle tension for years preceding his observations. In addition, they had lost considerable weight due to the lack of food. The relationship of prolonged starvation to blood creatin was pointed out previously (page 58) so the importance of this factor can not be overlooked. The effect of starvation in the author's experiments was adequately controlled.

Excluding the renal damage and increased muscle tension as major factors responsible for increases in blood creatin in cyanamid poisoning, the writer therefore ascribes the changes to a possible conversion of cyanamid to creatin or some other substance analysable as creatinin.

Changes in urine creatin-creatinin

The writer has earlier concluded that the fall in the excretion of creatinin after the injection of cyanamid is caused by failure of the kidneys. This is in agreement with some of the observations of Hesse (page 61).

The output of creatin decreased in rabbits No. 9, 12, and 13. In rabbit No. 4, however, the creatin excretion remained constant or even increased slightly. These results do not generally agree with that of Hesse, who observed a

rise in the excretion of creatin after the injection of cyanamid (page 63).

Changes in tissue creatin

An average rise of 4.4 per cent in muscle creatin was observed in the experiments with cyanamid. The various factors that might raise muscle creatin have been described on pages 67-80. The factors to be considered in the present experiments are starvation, spasms, fall in temperature, acidosis, and formation of creatin or stimulation of its formation.

The influence of starvation was eliminated by calculating the rises in muscle creatin from the values obtained on starved rabbits as controls.

The inconsistency in the changes in muscle creatin due to spasms was pointed out on page 70. Picrotoxin which produces intense spasms resulted in an average rise of only 2.8 per cent, whereas camphor, much less potent in this respect, produced rises of about 20 per cent. In the writer's experiments, spasms were observed but they were not of unusual intensity and frequency.

All of the rabbits except No. 9 exhibited a fall in body temperature. The increases in muscle creatin might thus be attributed to the accompanying drop in temperature (page 70).

The influence of acidosis can not be evaluated because determinations of the pH of the blood or urine were not per-

formed. Even if a condition of acidosis existed, it would not necessarily be responsible for a rise in muscle creatin since the presence of acidosis is accompanied by a fall in muscle creatin in nephrectomized rats.

It is thus difficult to consider the rise in muscle creatin after injection of cyanamid as due to a formation of creatin from cyanamid when the effects of body-temperature fall and possibly spasms also can not be satisfactorily disposed of.

The increases in the creatin content of liver and kidney although of great magnitude are probably not significant since the value for total liver and kidney creatin did not appreciably differ from the results obtained after ligation of the ureters.

Conclusions

The minimum lethal dose for the subcutaneous injection of pure cyanamid in mice was determined to be between 0.3 and 0.4 mgm. per gm. of body weight. This is in agreement with Coester's dose of 0.33 mgm. per gm.

The respiratory depression, hyper-excitability, paralysis, and spasms confirmed Coester's results. The gas and yellow fluid in the intestine, and diarrhea agreed with the findings of Koelsch, Stritt and Hesse with rabbits. Hemorrhages in the trachea were observed, in agreement with Koelsch's observations on rabbits.

The approximate minimum lethal dose for the intravenous injection of pure cyanamid in rabbits was determined to be between 0.15 and 0.30 gm. per kilo of body weight. This range of doses is smaller than Stritt's dose of 0.39 gm. and Hesse's dose of 0.4 gm. per kilo.

The respiratory depression, muscular weakness, paralysis, spasms, clonic movements of the extremities, and fall in body temperature agreed with the results of Coester, Stritt, Hesse, and Koelsch. The finding of gas and yellow fluid in the intestine confirmed the work of Stritt. The tracheitis was in agreement with the results of Stritt, Hesse, and Koelsch.

Microscopical examination of the mucosa of the intestine

revealed inflammatory and necrotic changes.

The average rise in blood creatinin in four rabbits about 24 hours after intravenous injections of lethal doses of pure cyanamid was 2.5 mgm. per 100 cc. (rise of 206 per cent). The average rise in blood creatin in the four experiments was 2.6 mgm. per 100 cc. (rise of 94 per cent). The increase in blood creatin was probably not due to kidney damage as was the case of increased creatinin, but more likely due to the conversion of cyanamid to creatin or a creatin-like substance.

The average rise in blood creatinin about 24 hours after ligation of the ureters in two rabbits was 9.95 mgm. per 100 cc. (rise of 603 per cent). The rise in blood creatin was 1.4 mgm. per 100 cc. (rise of 62 per cent).

There was no appreciable change in blood creatin and creatinin in two rabbits starved for about 24 hours.

The average hourly or total excretion of preformed creatinin fell slowly in three to nineteen hours after the injection of lethal doses of pure cyanamid in five rabbits. The fall in rate of excretion was 68 per cent. This was interpreted as evidence of interference with renal functions.

The average creatin excretion fell 48 per cent in five experiments.

Muscle creatin rose 4.4 per cent after intravenous injections of lethal doses of pure cyanamid in three rabbits.

The control values for muscle creatin were derived from two starved rabbits and from two rabbits which had both ureters ligated. These rises were due to changes in temperature, spasms, or conversion of cyanamid to a creatin-like substance.

Liver creatin rose 97.3 per cent in three rabbits injected with cyanamid. Kidney creatin rose 119 per cent in two rabbits. The control values were derived from the two starved animals. The rises were insignificant if the calculations were made with reference to the rabbits which had the ureters ligated. The increases in liver and kidney tissue were probably due to renal damage.

Summary

1. Pure cyanamid was prepared from a commercial preparation of cyanamid.
2. The minimum lethal dose for pure cyanamid subcutaneously injected in mice was determined to be between 0.3 and 0.4 mgm. per gm. of body weight.
3. The symptoms of poisoning in mice were confirmed.
4. The approximate minimum lethal dose for pure cyanamid intravenously injected in rabbits was found to vary between 0.15 and 0.30 gm. per kilo of body weight.
5. The symptoms of poisoning in rabbits were confirmed.
6. Blood creatinin rose 206 per cent about 24 hours after the intravenous injection of cyanamid in rabbits. This rise could be accounted by renal suppression.
7. Bloodcreatin rose 94 percent about 24 hours after the intravenous injection of cyanamid in rabbits. This rise was greater than could be accounted by renal suppression. It was ascribed to the conversion of cyanamid to creatin or a creatin-like substance.
8. Urinary creatinin and creatin fell considerably within 24 hours after the intravenous injection of cyanamid in rabbits. This was attributed to renal suppression.
9. Muscle creatin rose 4.4 per cent about 24 hours after the intravenous injection of cyanamid in rabbits.

The rise in muscle creatin could not be definitely attributed to a conversion of cyanamid to creatin.

10. Liver and kidney creatin rose 97.3 and 119 per cent respectively after the intravenous injection of cyanamid in rabbits. These rises were probably due to renal suppression.

Allinson, M. J. C., Henstell, H. H., and Himwich, H. E., The influence of glycin on creatinuria in peripheral neuritis. Am. Journ. Med. Sci., 1934, 188, 560-564.

Andes, J. E., The determination of blood creatine with 3,5-Dinitrobenzoic acid as a color reagent. Journ. Biol. Chem., 1937, Proc., 118, iv.

Baum, F., Über eine einfache Darstellung von reinem Cyanamid. Biochem. Zeitschr., 1910, 26, 325-332.

Baumann, L., and Hines, H. M., The origin of creatine. Journ. Biol. Chem., 1917, 31, 549-559.

Beard, H. H., and Barnes, B. O., The influence of feeding proteins, amino acids, and related substances upon creatine-creatinine metabolism. Journ. Biol. Chem., 1931, 94, 49-69.

Beard, H. H., and Boggess, T. S., The effect of parenteral injection of amino acids and related substances upon creatine formation and storage in the rat. Journ. Biol. Chem., 1936, 114, 771-782.

Behre, J. A., and Benedict, S. R., Studies in creatine and creatinine metabolism, IV. On the question of the occurrence of creatinine and creatine in blood. Journ. Biol. Chem., 1922, 52, 11-33.

Behre, J. A., and Benedict, S. R., On the presence of creatinine in blood. Journ. Biol. Chem., 1935, 110, 245-248.

Beilstein, Handbuch der organischen Chemie., 1921, 3, p. 74, 89, 91.

Benedict, S. R., and Behre, J. A., Some applications of a new color reaction for creatinine. Journ. Biol. Chem., 1936, 114, 515-532.

Bodansky, M., The effect of thyroid and thyroxine on the concentration of creatine in the heart, muscle, liver, and testes of the albino rat. Journ. Biol. Chem., 1935, 109, 615-622.

Bodansky, M., The influence of feeding amino acids and other compounds on the excretion of creatine and creatinine. Journ. Biol. Chem., 1936, 112, 615-624.

Bodansky, M., Comparison of glycine and guanidoacetic acid as precursors of creatine. Journ. Biol. Chem., 1936, 115, 641-652.

Bodansky, M., Schwab, E. H., and Brindley, P., Creatine metabolism in a case of generalized myositis fibrosa. Journ. Biol. Chem., 1930, 85, 307-325.

Boothby, W. M., Myasthenia gravis: second report on the effect of treatment with glycine. Proc. Staff. Meet. Mayo Clinic, 1932, 7, 737-756.

Brentano, C., Untersuchungen über die Entstehung der Kreatinurie. II Mitteilung: Die Beziehungen zwischen Kreatinurie und Muskelglykogen. Arch. exper. Pathol. u. Pharmakol., 1930, 155, 21-45.

Cameron, J. D. S., The effect of oral administration of creatinine on blood and urine. Journ. Exper. Physiol., 1933, 23, 351-366.

Cantarow, A., and Davis, R. C., Blood nonprotein nitrogen and creatinine in nephritis and prostatic obstruction. Journ. Lab. and Clin. Med., 1933, 18, 502-507.

Cardinale, G., and Arnone, G., La creatininemia in rapporto all'ipertonia muscolare di origine piramidale ed extra-piramidale. Il Policlinico, 1935, 42, 441-448.

Chanutin, A., (1) The effect of fasting and creatine ingestion upon the creatine and nitrogen content of the white rat. Journ. Biol. Chem., 1930, 87, Proc., viii-ix.

Chanutin, A., (2) Studies on the creatine and nitrogen content of the whole rat after the feeding of a variety of diets and after nephrectomy. Journ. Biol. Chem., 1930, 89, 765-774.

Chanutin, A., A study of the effect of creatine on growth and its distribution in the tissues of normal rats. Journ. Biol. Chem., 1927, 75, 549-557.

Chanutin, A., and Silvette, H., The influence of fasting and creatine feeding upon the creatine content of the tissues and blood of the white rat. Journ. Biol. Chem., 1928, 80, 589-608.

Chanutin, A., and Silvette, H., A study of creatine metabolism in the nephrectomized white rat. Journ. Biol. Chem., 1929, 85, 179-193.

Coester, E., Beitrag zur Kenntniss der Wirkung des Cyanamid. Kiel, A. F. Jensen, 1896, 8°, 24 pp.

Danielson, I. S., On the presence of creatinine in blood. Journ. Biol. Chem., 1936, 113, 181-195.

Denis, W., Creatine in human muscle. Journ. Biol. Chem., 1916, 26, 379-386.

Dittrich, J., Über die Cyanamidwirkung IV. Mitteilung: Zeitschr. ges. exper. Med., 1924, 43, 270-280.

Folin, O., Laboratory manual of biological chemistry. D. Appleton-Century Co., New York, 1934, 5th ed., vii, 367 pp.

Folin, O., and Denis, W., Protein metabolism from the standpoint of blood and tissue analysis, III. Further absorption experiments with special reference to the behavior of creatine and creatinine and to the formation of urea. Journ. Biol. Chem., 1912, 12, 141-162.

Folin, O., and Denis, W., On the creatinine and creatine content of blood. Journ. Biol. Chem., 1914, 17, 487-491.

Gaebler, O. H., Further studies of blood creatinine. Journ. Biol. Chem., 1930, 89, 451-466.

Gaebler, O. H., The apparent creatinine of serum and laked blood ultrafiltrates. Journ. Biol. Chem., 1937, 117, 397-413.

Gavrila, J., Creatinine et creatine du sang et du liquide céphalorachidien chez les sujets normaux et chez les néphrétiques azotémiques. Compt. rend. Soc. Biol., 1932, 109, 316-317.

Gergens, E., and Baumann, E., Über das Verhalten des Guanidin, Dicyandiamidin und Cyanamid im Organismus. Pflügers Arch. Physiol., 1876, 12, 205-214.

Glaubach, S., Über die Cyanamidvergiftung I Mitteilung: Untersuchungen über den chemischen Angriffspunkt des Cyanamids. Arch. exper. Pathol. u. Pharmakol., 1926, 117, 247-256.

Glaubach, S., Über die Cyanamidvergiftung II Mitteilung: Die Wirkung des Cyanamids auf Cystein und Cystin in Vitro. Arch. exper. Pathol. u. Pharmakol., 1926, 117, 257-265.

Goettsch, M., and Brown, E. F., Muscle creatine in nutritional muscular dystrophy of the rabbit. Journ. Biol. Chem., 1932, 97, 549-561.

Greenwald, I., and McGuire, G., The estimation of creatinine and of creatine in the blood. Journ. Biol. Chem., 1918, 34, 103-118.

Hamilton, A., Industrial poisons in the United States. MacMillan Co., New York, 1925, 1st ed., x, 590 pp.

Hawk, P. B., and Bergeim, O., Practical physiological chemistry. P. Blakiston's Son & Co., Inc., Philadelphia, 10th ed., 1931, xviii, 929 pp.

Hayman, J. M., Jr., Johnston, S. M., and Bender, J. A., On the presence of creatinine in blood. Journ. Biol. Chem., 1935, 108, 675-691.

Heidermanns, Abt. Zool. u. Physiol., 1927, 43, 223, quoted from Riesser, O. and Brentano, C. Arch. exper. Pathol. u. Pharmakol., 1930, 155, 1-20.

Heilbron, I. M., Dictionary of organic compounds, 1934, 1, p365.

Hellmuth, K., Reststickstoffuntersuchungen am Ende der Gravidität, unter der Geburt und im Wochenbett, mit besonderer Berücksichtigung der Graviditätstoxikosen. Arch. Gynäkol., 1923, 118, 18-58. Quoted from p. 99 of Hunter, A., 1928.

Hesse, E., Über die Cyanamidwirkung, I Mitteilung: Zeitschr. ges. exper. Med., 1921, 25, 321-344.

Hesse, E., Über die Cyanamidwirkung. II Mitteilung: Zeitschr. ges. exper. Med., 1922, 26, 337-351.

Higby, C. S., and Bowman, R. O., Report of two fatal cases. Journ. Am. Med. Assoc., 1934, 102, 1380.

Hope, Hanna, and Stallybrass, Industrial hygiene and medicine. London. Baillière, Tindall and Cox, 1923, p. 366, 501. Quoted from F. Schoofs. Journ. State Med., 1935, 43, 116.

Horn, F., Über das Verhalten des Zyanamids im Tierkorper. Zeitschr. Biol., 1933, 94, 159-170.

Hough, G., de. N., Progressive pseudohypertrophic muscular dystrophy. Journ. Bone and Joint Surg., 1931, 13, 825-847.

Hunter, A., Creatine and creatinine. London. Longmans, Green and Co., Ltd., 1928, vii, 281 pp.

International Critical Tables, 1926, 1, p. 176.

Januschke, Zeitschr. ges. exper. Med., 1913, 1, 129. Quoted from Hesse. Zeitschr. ges. exper. Med., 1921, 25, 321.

Jolliffe, N., and Colbert, C. N., The etiology of polyneuritis in the alcohol addict. Journ. Am. Med. Assoc., 1936, 107, 642-647.

Kácl, K., Der Einfluss der Muskelarbeit auf den Kreatin-und Kreatiningehalt im Blute normaler Menschen. Biochem. Zeitschr., 1932, 245, 452-458.

Koelsch, F., Über neuartige gewerbliche Erkrankungen in Kalkstickstoffbetrieben. Münch. Med. Wochnschr., 1914, 61, 1869-1870.

Koelsch, F., (1) Hautschädingungen durch Kalkstickstoff. Zentralbl. Gewerbehyg., 1916, 4, 103-107.

Koelsch, F., (2) Die Giftwirkung des Zyanamids. Zentralbl. Gewerbehyg., 1916, 4, 113-120.

Kühnau, J., Über den durch Wasserstoff abspaltbaren Schwefel der Erythrocyten und seine Beeinflussbarkeit durch Blausäure und Cyanamid. Klin. Wochnschr., 1927, 6, 406.

Kühnau, J., Über den durch Wasserstoff abspaltbaren Schwefel des Blutes und Insulins und sein Verhalten gegenuber Blausäure und Cyanamid. Arch. exper. Pathol. u. Pharmakol., 1927, 123, 24-49.

Langley, W. D., and Evans, M., The determination of creatinine with sodium 3,5-dinitrobenzoate. Journ. Biol. Chem., 1936, 115, 333-341.

Laurentier, C., Sur le zona généralisé et ses variétés gangréneuses (avec une note sur les toxicodermies par la cyanamide). Annal. Dermatol. et Syphiligr., 1925, 6, 134-147.

Linneberg, L. L., Et tilfelle av forgiftning med kalkkvelstoff. Norsk. Mag. laegevidenskap., 1933, 94, 734-736.

Looney, J. M., (1) A biochemical study of the blood in mental disorders. Am. Journ. Psychiatr., 1924, 4, 29-39.

Looney, J. M., (2) The relation between increased muscle tension and creatine. Am. Journ. Physiol., 1924, 69, 638-644.

Löw, Chem. Zeitung., 1908, 676. Quoted from Stutzer and Söll, 1910.

Mann, L., Klin. Wochnschr., 1928, 7, 569-570. Vereining. sudostdeut. Psychiatr. u. Neurol. Breslau. Extract from report of meeting.

Mattice, M. R., Chemical procedures for clinical laboratories. Lea and Fibiger, Philadelphia, 1st ed., 1936, 520 pp.

Mendel, L. B., and Rose, W. C., Experimental studies on creatine and creatinine, II. Inanition and the creatine content of muscle. Journ. Biol. Chem., 1911, 10, 225-264.

Meyer, C. E., and Rose, W. C., Arginine metabolism, II. The relation of the arginine content of the diet to the creatine-creatinine production during growth. Journ. Biol. Chem., 1933, 102, 461-471.

Monaco, D. L., and Prattali, L., Sull'azione della calciocianamide. Arch. farmacol. sperim. e sci., 1918, 26, 179-192.

Morgulis, S., and Edwards, A. C., Chemical changes in the blood during fasting and subsequent refeeding. Experiments on dogs. Amer. Journ. Physiol., 1924, 68, 477-498.

Morgulis, S., and Young, A., Metabolism in myotonia atrophica. Arch. Intern. Med., 1931, 48, 569-575.

Myers, V. C., and Fine, M. S., The influence of the administration of creatine and creatinine on the creatine content of muscle. Journ. Biol. Chem., 1913, 16, 169-186.

Myers, V. C., and Fine, M. S., The influence of starvation upon the creatine content of muscle. Journ. Biol. Chem., 1913, 15, 283-304.

Ohnsorge, Kalkstickstoff-Cyanamidvergiftung. Klin. Wochnschr., 1928, 7, 2224.

Osterberg, A. E., and Kendall, E. C., The preparation of cyanamide. Journ. Biol. Chem., 1917, 32, 297-298.

Putseys, F., and Swaen, A., Über die physiologische Wirkung des Schwefel-sauren Guanidins. Pflügers Arch. Physiol., 1876, 112, 597

Raida, H., Über die Cyanamidwirkung. III Mitteilung: Quantitative Cyanamidbestimmungen in Geweben, Zeitschr. ges. exper. Med., 1923, 31, 215-220.

Rakestraw, N. H., Chemical factors in fatigue I. The effect of muscular exercise upon certain common blood constituents. Journ. Biol. Chem., 1921, 47, 565-590.

Riesser, O., Theoretisches und Experimentelles zur Frage der Kreatinbildung im tierischen Organismus. Zeitschr. Physiol. Chem., 1913, 86, 415-453.

Riesser, O., Über Tonus und Kreatingehalt der Muskeln in ihren Beziehungen zu Wärmeregulation und Zentral-sympathischer Erregung. Arch. exper. Pathol. u. Pharmakol., 1916, 80, 183-230.

Riesser, O., und Brentano, C., Untersuchungen über die Entstehung der Kreatinurie. I Mitteilung: Azidose und Kreatinurie. Arch. exper. Pathol. u. Pharmakol., 1930, 155, 1-20.

Rigo, L., and Frey, K., Über den Einfluss des Insulins und Adrenalins auf den Kreatinin-Kreatin-und Phosphorsäure gehalt des Blutes. Arch. Exper. Pathol. u. Pharmakol., 1934, 175, 8-13.

Rose, W. C., The metabolism of creatine and creatinine. Ann. Rev. Biochem., 1935, 4, 243-262.

Rose, W. C., Helmer, O. M., and Chanutin, A., A modified method for the estimation of total creatinine in small amounts of tissues. Journ. Biol. Chem., 1927, 75, 543-548.

Rosengarten, F., and Strecker, A., Über die Spaltung des Caffeidins durch Barythydrat. Ann. Chem. Pharm., 1871, 157, 1-6. Quoted from p. 7 of Hunter, A., 1928.

Schoofs, F., Toxicity of calcium cyanamide. Journ. State Med., 1935, 43, 116-118.

Selman, J. J., and Linegar, C. R., A case of marked creatinemia with recovery. Journ. Lab. & Clin. Med., 1933, 18, 1032-1034.

Strecker, A., Jahresber. Fortsch. Chem. für 1886, 686 (footnote). Quoted from p. 7 of Hunter, A., 1928.

Stritt, W., Über die Giftwirkungen der als Dungemittel verwandten Cyanverbindungen und ihrer Zersetzungsprodukte. Zeitschr. Hyg. u. Infekt., 1909, 62, 169-198.

Stutzer, A., and Söll, J., Tierphysiologie Versuche über die Wirkung von Cyanamid und von einigen seiner Umsetzungsprodukte. Biochem. Zeitschr., 1910, 25, 215-219.

Sullivan, M. X., A colorimetric test for guanidine. Proc. Soc. Exper. Biol. and Med., 1935, 33, 106-108.

Thompson, W. H., The metabolism of arginine, III. Arginine and creatine formation. Journ. Physiol., 1917, 51, 111-153.

Van Husen, Hautentzündung durch Kalkstickstoffdunger. Münch. Med. Wochnschr., 1919, 66, 750.

Van Itallie, L., en U G. Bylsma, Toxicologie en gerechtelyke Scheikunde. Amsterdam. Centen., 1928, 251. Quoted from F. Schoofs, Journ. State. Med., 1935, 43, 116.

Volhard, J., Über die Synthese des Kreatins. Sitzungsber. Königl. Bayer. Akad. Wiss., 1868, 2, 472. Quoted from Hunter, A., 1928.

Walker, B. S., and Huntsinger, M. E., The phosphorus partition in normal whole blood. Journ. Lab. & Clin. Med., 1930, 16, 247-255.

Wang, C. C., and Dentler, M. L., Creatinine and creatine in the blood. Journ. Biol. Chem., 1920, 45, 237-243.

Wishart, G. H., The effect of injection of guanidine on the creatine content of muscle. Journ. Physiol., 1920, 53, 440-445.

Zappacosta, M., Sul significato della ipercreatinemia nella ipertensione essenziale. La Riforma Med., 1935, 51, 1430-1436.

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Acknowledgment

The author wishes to thank Miss Etta Bloom for her kindness in taking the electrocardiographs. He also wishes to express thanks to the Department of Pathology for preparing the microscopical sections.